Katherine E. Reed, Ph.D.
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8EHQ - 0404 - 14523

AR 274415

Certified Mail

March 30, 2004

Document Processing Center
EPA East – Room 6428 Attn: Section 8(e)
Office of Pollution Prevention and Toxics
US EPA
1200 Pennsylvania Avenue NW
Washington DC 20460-0001

RE: TSCA 8(E) SUPPLEMENTAL SUBMISSION:
Docket No. 8EHQ-1000-4523

Dear Docket Coordinators:

3M previously informed the EPA (September 28, 2000, and September 19, 2001) of the results of 4-hour and 28-day inhalation studies in rats conducted with perfluorobutanesulfonyl fluoride (PBSF, CAS# 375-72-4) in which neurotoxicity effects were observed. The testing facility that conducted this work, Huntingdon Life Sciences Ltd., recently provided 3M with a final report for the 5-day inhalation rangefinder study that had been conducted in conjunction with the definitive acute and subchronic studies.

In today's submission, 3M is providing EPA with the results of the 5-day inhalation rangefinder study for PBSF. The 5-day study indicates neurotoxicity effects consistent with the previously reported results. Enclosed please find the following final report:

- Preliminary Toxicity Study by Repeat Dose Inhalation Administration to CD Rats for 5 Days

Please contact Paul Lieder (651-737-2678) if you have any questions or if we can provide additional information.

Sincerely,

CONTAINS NO CBI

Katherine E. Reed

Katherine E. Reed
Staff Vice President
Environmental, Health and Safety Operations

Enclosure

T-7499

PRELIMINARY TOXICITY STUDY BY REPEAT DOSE INHALATION ADMINISTRATION TO CD RATS FOR 5 DAYS

T-7499

PRELIMINARY TOXICITY STUDY BY REPEAT DOSE INHALATION ADMINISTRATION TO CD RATS FOR 5 DAYS

Sponsor

3M Medical Department,
Toxicology Services,
3M Center Building 220-2E-02,
P.O. Box 33220,
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USA.

Research Laboratory

Huntingdon Life Sciences Ltd.,
Woolley Road,
Alconbury,
Huntingdon,
Cambridgeshire,
PE28 4HS,
ENGLAND.

Report issued: 17 June 2002

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid:

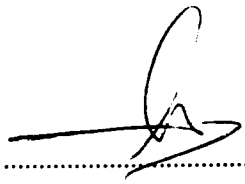
The UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No 3106).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.

EC Commission Directive, 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

United States Environmental Protection Agency, (TSCA) Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Information regarding test substance characterisation, namely the batch number and expiry date, was not available to Huntingdon Life Sciences for compliance with the Good Laboratory Practice regulations given above.



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17 June 2002

Derek W. Coombs, B.Sc., M.Sc.,
Study Director,
Huntingdon Life Sciences Ltd.

Date

.....

.....

Sponsor,
3M Toxicology Services.

Date

.....

.....

Submitter.

Date

QUALITY ASSURANCE STATEMENT

The following have been inspected or audited in relation to this study:

Study Phases Inspected	Date of Inspection	Date of Reporting
Protocol audit	6 July 2000	6 July 2000
Study based inspections		
Study preparation Exposure procedure Test substance records Clinical signs Exposure sampling Records audit	10 July 2000	10 July 2000
Cholinesterase bleed	14 July 2000	17 July 20000
Post mortem Records audit	17 July 2000	17 July 2000
Report audit	26 September 2000	28 September 2000

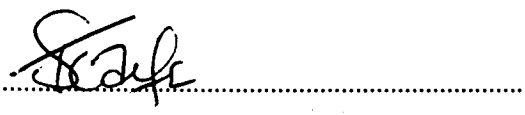
Protocol: An audit of the protocol for this study was conducted and reported to the Study Director and Company Management as indicated above.

Study based inspections: Inspections and audits of phases of this study were conducted and reported to the Study Director and Company Management as indicated above.

Process based inspections: At or about the time this study was in progress inspections and audits of other routine and repetitive procedures employed on this type of study were carried out. These were promptly reported to appropriate Company Management.

Report Audit: This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Study Director and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results to reflect the raw data.



14 June 2002

Tracy Scarfe, F.R.Q.A.,
Group Manager,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

Date

CONTRIBUTING SCIENTISTS

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Director of Pathology.

CONTENTS

APPENDICES

SUMMARY

Three groups of rats (each of 5 males and 5 females) of the Crl:CD® BR strain were exposed to T-7499, 6 hours a day for 5 consecutive days using a whole-body exposure system. A fourth group, acting a control, was exposed to air only.

The study mean analysed concentrations of T-7499 were 203, 345 and 507 ppm for the Low, Intermediate and High dose groups respectively.

The following comments are made in summary:

Clinical signs observed during exposure included initial restless behaviour, circling movement ('chasing tail') and 'mouthing' of tails, which were followed by lethargy, hunched posture and piloerection. Slow and exaggerated breathing were also evident during exposure.

Clinical signs observed immediately post exposure included slow, exaggerated and noisy ('scratchy') breathing, sensitivity to touch and piloerection. On Day 1 only, the normal breathing rate of a test rat was noted to slow and an audible 'pop' was then evident followed by a return to a normal breathing rate.

All of the above signs had resolved prior to exposure the following day.

The mean bodyweight gain for all test groups was statistically significantly lower than controls.

A reduction in food consumption was evident for Groups 3 and 4 male rats.

The water consumption and cholinesterase activity were considered not to be affected by treatment.

Necropsy revealed no treatment-related macroscopic findings and no treatment-related differences in organ weights.

Histopathological examination of the respiratory tract revealed no treatment-related findings.

Conclusion

In view of the clinical and bodyweight findings summarised above, target concentrations of 50, 150 and 450 ppm are considered suitable for a subsequent 28-day study.

INTRODUCTION

The purpose of this study performed at Huntingdon Life Sciences Limited, Huntingdon, England was to assess the systemic toxic potential in rats to repeat administration by inhalation, in whole-body exposure chambers, of the test substance T-7499, for 5 consecutive days.

The results of this study will be used to select exposure levels for a subsequent 28-day inhalation toxicity study.

The test substance was administered by inhalation, a possible route for accidental exposure in man. The rat was the species of choice due to regulatory requirements and the strain was selected on account of the availability of comprehensive background data, relating to clinical and pathological parameters, at our laboratories.

Exposure levels were selected on the basis of consultation with the Sponsor and on the results obtained from an acute inhalation toxicity study (HLS study number MIN 244/003594).

The in-life phase of the study was undertaken between 10 and 17 July 2000.

RELEVANT STUDY DATES

Approved by:

Study Director	30 June 2000
HRC Management	30 June 2000
Study Sponsor	6 July 2000

Animals arrived at HRC:	28 June 2000
Allocation to groups:	28 June 2000
Exposures commenced:	10 July 2000

Cholinesterase activity (sampling):

Day 5 (Blood)	14 July 2000
Day 8 (Brain)	17 July 2000

Terminal kill:

Day 8	17 July 2000

Experimental completion date:	14 August 2000

TEST SUBSTANCE

Tradename:	T-7499
Chemical name:	Perfluorobutyl sulfonyl fluoride
Other name:	PBSF
Intended use:	None stated
Appearance:	Clear colourless liquid (presented in a steel pressure vessel)
Storage conditions:	Ambient temperature or in a refrigerator, unless otherwise stated by the Sponsor
Amount received:	*Ca*. 35 kg
Batch number:	None stated
Assay:	Perfluorobutyl sulfonyl fluoride 96-98% Perfluorosulfolane 2-4%
Expiry date:	None stated
Date received:	26 April 2000
Supplier:	Sponsor

EXPERIMENTAL PROCEDURE

ANIMALS

Fifty (25 male and 25 female) rats, aged approximately 6 weeks, of the Crl:CD® BR, a caesarean derived strain of Sprague-Dawley origin, were obtained from Charles River (UK) Limited, Manston Road Margate, Kent, on 28 June 2000.

On arrival, all animals were examined for abnormalities and signs of overt ill health and randomly allocated to 1 of 4 groups, each of 5 males and 5 females. The animals were then uniquely identified by numbers tattooed into the ear pinnae. The animals were acclimatised for at least 7 days before commencement of treatment.

The identification of individual rats in the 4 groups were as follows:

Group	Rat numbers	
	M	F
1 (Control)	1 - 5	21 - 25
2 (Low dose)	6 - 10	26 - 30
3 (Inter. dose)	11 - 15	31 - 35
4 (High dose)	16 - 20	36 - 40
Reserve	A - E	F - J

The remaining animals, 5 males and 5 females, were assigned to the reserve group. These were identified by a letter written on the tail and were retained as potential replacements during the acclimatisation period. Following the commencement of exposures the reserves were killed.

ACCOMMODATION

The rats were housed 5 of the same sex to a cage in suspended stainless steel cages fitted with mesh front, back and floor with stainless steel sheet sides. The cages were suspended on racks. Plastic trays lined with absorbent paper were placed below each cage to collect animal excreta and the paper was changed daily. Each cage had a coloured label identifying the group and the numbers of the animals contained within it. The rats were kept in a single room and, additionally, after the start of the exposure period, each group was positioned on an individual cage battery. Each battery was in a separate ventilated cabinet within the holding room in order to avoid the possibility of inhalation of test material from the rats in other groups. Exposure took place in the same room.

The temperature and relative humidity of the holding room were recorded using a Kent Clearspan recorder. The study holding room temperature and relative humidity were set to be maintained within limits of 21 ± 2°C and 55 ± 10% respectively. Recorded ranges were 20.5 to 21.5°C and 49 to 57% relative humidity.

Lighting was controlled to give 12 hours light (0600 - 1800 hours) and 12 hours dark per 24 hours.

DIET

While in their cages, all rats had access to a weighed quantity of standard quality-controlled laboratory rat food (SDS Rat and Mouse No. 1 SQC modified maintenance diet, Special Diets Services, Witham, Essex).

There was no information available to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet. The analytical data have been lodged in Huntingdon Life Sciences Archives.

Tap water was available from moulded polypropylene water bottles at all times while the rats were in the cages. The water bottles were emptied and refilled daily during the study.

There was no information available to indicate that any substance likely to influence the effect of the test system could reasonably be expected to be present in the drinking water.

Results of the routine physical and chemical analyses of water at source (sampling point, Grafham Final Water) as conducted by the supplier, Anglian Water Services Ltd, have been made available to Huntingdon Life Sciences. Anglian Water takes its guidelines on water quality from the EEC directive relating to water for human consumption, *viz.* Council Directive 80/778/EEC.

The analytical data have been lodged in Huntingdon Life Sciences Archives.

ADMINISTRATION

The vaporous test substance was administered for 6 hours a day, for 5 consecutive days.

The rats were exposed to the control/test atmosphere in whole-body exposure chambers constructed from stainless steel and glass, with an internal volume of 0.75 m^3. The test atmosphere was produced by metering the test liquid from polypropylene syringes to a glass vaporiser and diluted with clean air prior to the resultant vapour atmosphere passing into the exposure chamber.

The target concentrations for exposure were 200 ppm (Low dose), 350 ppm (Intermediate dose) and 500 ppm (High dose). Control rats received air only.

Details of administration and analysis of the test atmospheres together with the results obtained are presented in **ADMINISTRATION OF T-7499 BY INHALATION TO RATS** appended to this report.

CLINICAL INVESTIGATIONS

Dated and signed records of all activities relating to the day to day running and maintenance of the study, as well as to the group observations and examinations outlined in this procedure were recorded in the Study Daybook. In addition, observations relating to individual animals made throughout the study were recorded.

Mortality

Throughout the study, all cages were checked in the morning and again at the end of the normal working day for dead or moribund animals.

Clinical signs

Dated and signed records of appearance, change and disappearance of clinical signs were maintained. Individual animal records were maintained on the basis of:

- any observation, considered to be of possible importance, made at any time during the study;
- any observation, considered to be of possible importance, made during transfer to exposure cages (prior to exposure), on return to holding cages (after exposure) and during daily checks;
- a careful external examination made daily commencing one-week prior to the start of exposures when special attention was given to the detection of audible respiratory sounds.

During exposure signs were recorded as a group response where all visible animals appeared to be responding similarly to the test substance.

BODYWEIGHT

Each rat was weighed on arrival following random allocation. The weight of each rat was recorded daily, commencing 1 week prior to the start of exposure. During the treatment period, bodyweight was recorded before exposure. Bodyweight was also recorded prior to necropsy.

FOOD CONSUMPTION

The quantity of food consumed by each cage of rats was recorded daily, commencing 1 week prior to the start of exposures until the end of the study.

WATER CONSUMPTION

The quantity of water consumed by each cage of rats was recorded daily, commencing 1 week prior to the start of exposures until the end of the study.

LABORATORY INVESTIGATIONS

Sample collection

Blood samples for cholinesterase activity were collected from all group animals immediately following exposure on Day 5.

Samples of venous blood were withdrawn from the retro-orbital sinus using sterile glass pipettes while the rats were held under isoflurane anaesthesia.

The blood samples collected were put into tubes containing the following anticoagulant:

Heparin - for cholinesterase activity investigations (0.5 ml whole blood)

Cholinesterase activity in plasma and erythrocytes

Immediately following the end of the exposure on Day 5, samples of whole blood were removed from the retro-orbital sinus of each rat, while lightly anaesthetised with isoflurane.

The whole blood samples were placed in heparinised containers, mixed briefly and chilled in iced water prior to analysis of cholinesterase activity to minimise reversibility of any cholinesterase inhibition. Samples were immediately transferred to Central Laboratory Services (CLS) for processing.

Samples of whole blood were centrifuged for 10 minutes at 3000 rpm prior to preparation of sample for plasma and erythrocytes (Red Blood Cells, RBCs). Samples were stored deep frozen (-70°C) pending assay for cholinesterase activity. Samples from Groups 1 and 4 were analysed using a modification of the method of Ellman, G.L *et al* (1961) and expressed as U/L. Samples from Groups 2 and 3 were stored for approximately 1 month for possible analysis. All samples, including residues for Groups 1 and 4, were subsequently returned to the Sponsor. Any further processing and analysis of the samples and data collation will be the responsibility of the Sponsor.

The exposures on this day were staggered by approximately 15 minutes between each group in order that blood could be removed in batches of 10 samples for immediate transfer to CLS to minimise the delay between sampling and analysis.

The cholinesterase activity investigations performed are listed below, together with an abbreviated title (for use in appendices and tables), methods and the units of measurement.

The following estimations were performed using a modified Ellman assay:

	Units
Plasma cholinesterase activity (Plasm CHE)	U/L
Red blood cell cholinesterase activity (RCHE DTNB)	U/L

TERMINAL STUDIES

Sacrifice

All groups were killed following 5 consecutive days of exposure. The terminal kill was performed on Day 8 of the study.

Animals were killed by an intraperitoneal injection of pentobarbitone sodium followed by exsanguination from the brachial arteries.

Macroscopic examination and organ weights

All rats were subjected to a detailed macroscopic examination.

The following organs from all animals killed at the scheduled sacrifice were dissected free of fat and weighed:

adrenals	kidneys
brain (left half)‡	liver
epididymides	lungs
heart	testes

‡ for determination of cholinesterase activity

Bilateral organs were weighed together.

Brain cholinesterase activity

At necropsy the left half of the brain was weighed, frozen immediately in a cardice/hexane mixture and stored deep frozen (-70°C) pending assay for cholinesterase activity. Immediately prior to analysis batches of up to 10 were thawed from deep frozen to 1 - 4°C and homogenised. This procedure was undertaken in order to minimise reversal of any inhibition that may have occurred between removal and analysis. Samples from Groups 1 and 4 were analysed using a modification of the method of Ellman, G.L *et al* (1961) and expressed as U/Kg. Samples from Groups 2 and 3 were stored for approximately 1 month for possible analysis. All samples, including residues for Groups 1 and 4, were subsequently returned to the Sponsor. Any further processing and analysis of the samples and data collation will be the responsibility of the Sponsor.

The cholinesterase activity investigation performed is listed below, together with an abbreviated title (for use in appendices and tables), methods and the units of measurement.

The following estimation as performed using a modified Ellman assay:

	Units
Brain cholinesterase activity (Brain CHE)	U/Kg

Fixation of tissues

Samples, or the whole, of the following organ/tissues, together with any macroscopically abnormal entities were preserved in 10% neutral buffered formalin, except the brain (left half), which was frozen in a cardice/hexane mixture as described above, the eyes, which were preserved in Davidson's fixative, and testes and epididymides, which were fixed in Bouin's solution and then transferred to 70% alcohol.

abnormalities*
adrenals
alimentary tract
  oesophagus
  stomach
  duodenum
  jejunum
  ileum
  caecum
  colon
  rectum
animal identification mark
aorta (thoracic)
brain (left half) [a]
brain (right half)
bronchi
epididymides
eyes
femur
harderian gland
head [b]
heart
kidneys
lachrymal glands
larynx (2 levels)*
liver
lungs (all lobes)*
lymph nodes (mandibular, mesenteric and tracheobronchial)
mammary area (caudal)
nasal turbinates (3 levels)*
optic nerves
ovaries
pancreas
pituitary
prostate
pharynx
salivary glands
sciatic nerve
seminal vesicles
skeletal muscle (thigh)
skin
spinal cord
spleen
sternum
testes
thymus
thyroids (with parathyroids)
tongue
trachea (including bifurcation)*
urinary bladder
uterus (with cervix)
vagina

[a] Tissue frozen pending analysis of cholinesterase activity

[b] The nasal cavity was flushed with a fixative prior to immersion. The remaining head was retained for nasal cavity, paranasal sinuses and nasopharynx

Histopathology

Histopathological examinations were performed on all scheduled tissues (marked with *) for Groups 1 and 4. These tissues were embedded in paraffin wax and sections approximately 4-5 µm thick were cut, processed and stained with haematoxylin and eosin for examination by light microscope.

Histopathological examination was only performed on any abnormal tissues arising from Groups 2 and 3.

STATISTICAL ANALYSIS

All statistical analyses were performed separately for males and females.

For all parameters the analyses were performed using the individual animal as the experimental unit. Bodyweight data were analysed using weight gains. The following sequence of statistical tests was used for bodyweight, organ weight and clinical pathology data.

> If the data consist predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analysed by appropriate methods. Otherwise:
>
> Bartlett's test was applied to test for heterogeneity of variance between treatments; where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.
>
> If no significant heterogeneity was detected (or if a satisfactory transformation was found), and more than two groups were being compared, group means were compared using Williams' test for a dose-related response (Williams, 1971 and 1972), or if there was evidence for a non-monotonic response, Dunnett's test (Dunnett, 1955 and 1964). For separate two-group comparisons, a Student's *t*-test was used.
>
> If significant heterogeneity of variance was present (and could not be removed by a logarithmic transformation), groups were compared using Shirley's non-parametric test for a dose-related response (Shirley, 1977), or if there was evidence for a non-monotonic response, Dunn's test (Dunn, 1964). For separate two-group comparisons, a Wilcoxon rank sum test (Wilcoxon, 1945) was used.

Where appropriate, analysis of covariance was used in place of analysis of variance in the above sequence. For organ weight data, the final bodyweight was used as covariate in an attempt to allow for differences in bodyweight which might influence the organ weights.

For microscopic findings Fisher's exact test was employed to detect treatment-related differences.

LOCATION OF STUDY RECORDS

All raw data, samples and specimens arising from the performance of this study will remain the property of the Sponsor.

Types of sample and specimen that are unsuitable, by reason of instability, for long term retention and archiving may be disposed of after the periods stated in Huntingdon Life Sciences, Standard Operating Procedures.

All other samples and specimens and all raw data will be retained by Huntingdon Life Sciences in its archive for a period of five years from the date on which the Study Director signs the final report. After such time, the Sponsor will be contacted and his advice sought on the return, disposal or further retention of the materials. If requested, Huntingdon Life Sciences will continue to retain the materials subject to a reasonable fee being agreed with the Sponsor.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Chamber analysed concentration of T-7499

The data are presented in **ADMINISTRATION OF T-7499 BY INHALATION TO RATS**

The data are summarised below:

Group	Chamber concentration (ppm)		
	Target	Analysed	
		Mean	sd
2 (Low dose)	200	203	4.2
3 (Inter. dose)	350	345	38.2
4 (High dose)	500	507	24.1

The analysed concentrations were in good agreement with the target concentrations.

CLINICAL OBSERVATIONS

Mortality

There were no unscheduled deaths.

Clinical signs

The data are presented as follows:

Table 1	during exposure – group distribution of observations
Table 2	post exposure – incidence summary
Appendix 1	post exposure – individual observations
Appendix 2	daily – individual observations

Clinical signs observed during exposure included initial restless behaviour for Groups 3 and 4 on Day 1. Circling movement ('chasing tail') and 'mouthing' of tails were also evident during this period for Group 4 rats. Lethargy was evident for all test groups during each exposure (Days 1 to 5). Hunched posture was evident during exposure for Groups 3 and 4 from Days 2 to 4. Piloerection was noted for Group 4 rats on Day 2. Slow and exaggerated breathing was noted on Days 2 to 3 for Group 4 rats and on Day 3 for Group 3. Breathing was exaggerated only on Day 4 for Groups 3 and 4.

Clinical signs observed immediately post exposure included slowing of the normal breathing rate followed by an audible 'pop' and then a return to a normal breathing rate for a Group 4 male rat on Day 1. Slow and exaggerated breathing and piloerection were noted following exposure for a proportion of Group 4 rats on Day 2. Noisy ('scratchy') breathing and sensitivity to touch were evident following exposure for a proportion of Groups 3 and 4 on Days 2 to 5. These later two signs were also evident following exposure for a proportion of Groups 2 rats on Days 4 and 5 respectively.

All the above signs had resolved prior to examination the next day.

At other times, a dry skin abrasion to tail, and dry, opaque or prominent left eyes were observed. The observations for the eyes are considered associated with the peri-orbital sinus bleeding performed on the rats following exposure on Day 5. These signs are considered incidental and not related to treatment.

Bodyweight

The data are presented as follows:

Figure 1 - group mean values (g)
Table 3 - group mean values (g)
Appendix 3 - individual values (g)

The mean bodyweight gain for all test groups (Days 0 to 7) was statistically significantly lower than controls.

Group mean bodyweight losses were evident for Groups 3 and 4 male rats and Group 2 female rats following exposure on Day 1. Thereafter, mean bodyweight losses or reduced gains, compared with controls, were evident intermittently for all test groups, particularly Group 4 (High dose).

Food consumption

The data are presented as follows:

Table 4 - group mean values (g/animal)

A reduction in food consumption was evident for Groups 3 and 4 male rats.

The food consumption of Group 2 males and test females was considered not to be affected by treatment.

Water consumption

The data are presented as follows:

Table 5 - group mean values (g/animal)

There were no treatment-related findings.

LABORATORY INVESTIGATIONS

Cholinesterase activity

The data are presented as follows:

Table 6	- group mean values
Appendix 4	- individual values

There were no treatment-related findings.

TERMINAL STUDIES

Macroscopic pathology

The data are presented as follows:

Table 7	- incidence summary
Appendix 6	- individual pathological findings

There were no treatment-related findings.

Organ weights

The data are presented as follows:

Table 8	- group mean values (g)
Appendix 5	- individual values (g)

There were no treatment-related findings.

Microscopic pathology

The data are presented as follows:

Table 9	- expanded incidence summary
Appendix 6	- individual pathological findings

Treatment related findings

There were no microscopic findings which were considered to be related to treatment with T-7499.

Incidental findings

All microscopic findings were considered to be incidental and of no toxicological importance.

DISCUSSION

In this study, rats were exposed by inhalation to the vapour of T-7499 for 6 hours a day, for 5 consecutive days. The study mean exposure levels were 203, 345 and 507 ppm.

Clinical signs during exposure may be considered consistent with a neurotoxic effect similar to limited inhibition of cholinesterase activity, namely initial restless behaviour (on Day 1) at 345 and 507 ppm and lethargy at all exposure levels. However, there was no treatment-related effect on the cholinesterase activity (plasma, erythrocytes and brain) of High dose rats.

The overall mean bodyweight gain for all test groups was lower than controls. A reduced food consumption was also evident for male animals in Groups 3 and 4.

There was no treatment-related effect on water consumption and no treatment-related findings were evident at necropsy. Histopathological examination of the respiratory tract revealed no treatment-related findings.

In view of the clinical and bodyweight findings summarised above, target concentrations of 50, 150 and 450 ppm are considered suitable for a subsequent 28-day study.

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ELLMAN, G.L., COURTNEY, K.D, ADRES, V and FEATHERSTONE, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**, 88.

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SHIRLEY, E. (1977) A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics*, **33**, 386.

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WILLIAMS, D.A. (1972) The comparison of several dose levels with a zero dose control. *Biometrics*, **28**, 519.

FIGURE 1

Bodyweights – group mean values (g)

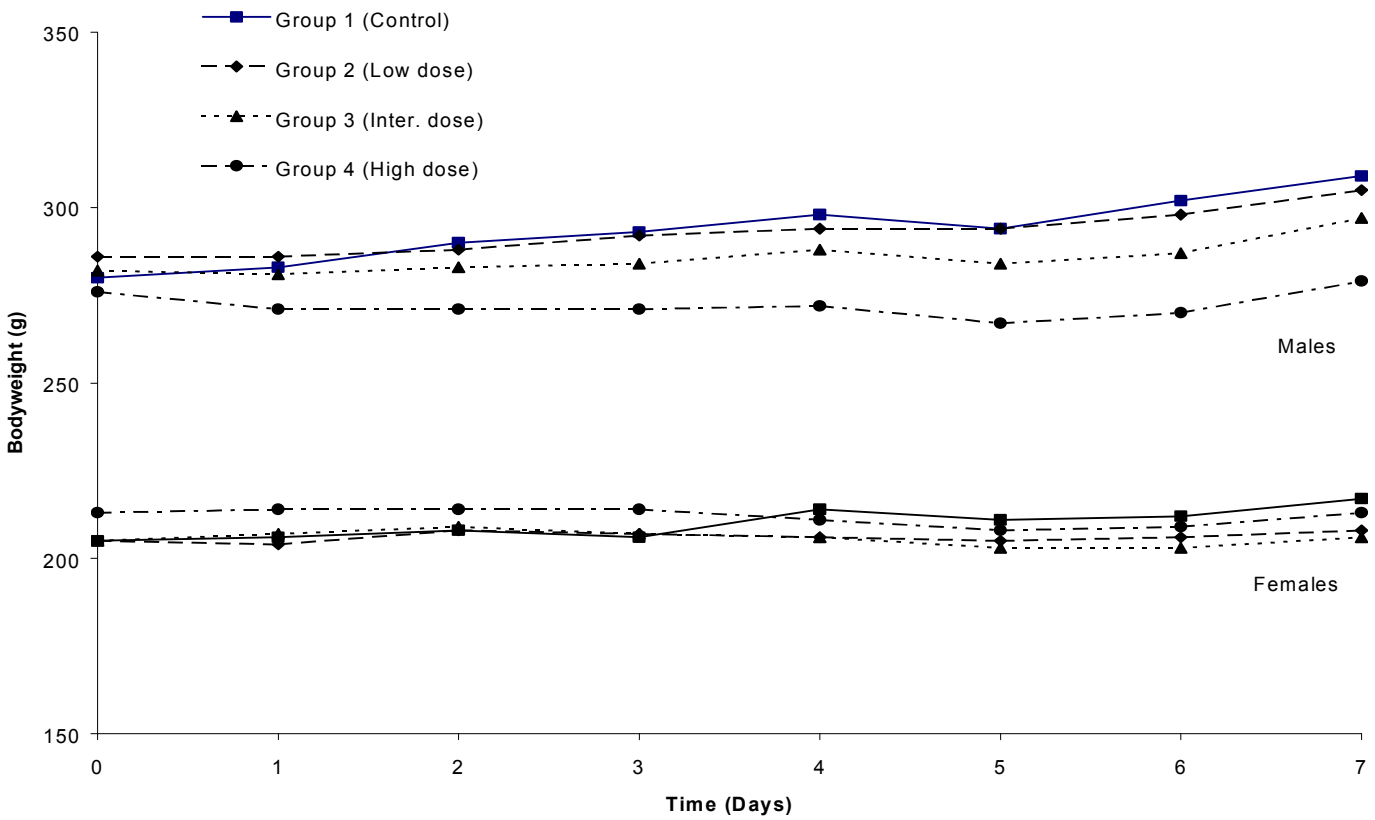

TABLE 1

Clinical signs during exposure – group distribution of observations

Group	Sign	Exposure number				
		1	2	3	4	5
1 (Control)	No abnormalities detected	√	√	√	√	√
2 (Low dose)	Lethargic	√	√	√	√	√
3 (Inter. dose)	Restless behaviour	√				
	Lethargic	√	√	√	√	√
	Hunched posture		√	√	√	
	Slow and exaggerated breathing			√		
	Exaggerated breathing				√	
4 (High dose)	Restless behaviour	√				
	Circling movement	√				
	Mouthing tail	√				
	Lethargic	√	√	√	√	√
	Hunched posture		√	√	√	
	Pilo-erection		√			
	Slow and exaggerated breathing		√	√		
	Exaggerated breathing				√	

TABLE 2

Clinical signs post exposure – incidence summary

Group	Sign	Number showing sign				
		Exposure number				
		1	2	3	4	5
1M (Control)	No abnormalities detected	5	5	5	5	5
2M (Low dose)	No abnormalities detected	5	5	5	4	5
	Noisy breathing (scratchy)				1	
3M (Inter. dose)	No abnormalities detected	5	3	5	3	2
	Sensitive to touch		1		2	
	Noisy breathing (scratchy)		1		2	3
4M (High dose)	No abnormalities detected	4		4	4	2
	Pilo-erection		4			
	Noisy breathing (scratchy)			1	1	3
	Breathing rate slows followed by an audible 'pop'	1				
	Slow and exaggerated breathing		4			
1F (Control)	No abnormalities detected	5	5	5	5	5
2F (Low dose)	No abnormalities detected	5	5	5	5	3
	Sensitive to touch					2
3F (Inter. dose)	No abnormalities detected	5	1	2	2	2
	Sensitive to touch		2	3	3	3
	Noisy breathing (scratchy)		3		1	2
4F (High dose)	No abnormalities detected	5		1	3	4
	Sensitive to touch		5	3	1	
	Noisy breathing (scratchy)		4	4	1	1
	Slow and exaggerated breathing		3			

TABLE 3

Bodyweight - group mean values (g)

Print No: 0026

Printed: 04-AUG-00

Xybion protocol number: MIN 251

GROUP	:	1	2	3	4				
COMPOUND	:	CONTROL	------	T-7499	-----				
DOSAGE (PPM)	:	0	200	350	500				
SEX:		MALE				FEMALE			
GROUP:		1	2	3	4	1	2	3	4
DAY									
-7		224	225	225	220	179	183	180	189
-6		239	241	242	236	187	192	188	199
-5		248	248	251	243	190	194	190	200
-4		255	250	251	244	189	193	190	199
-3		262	264	263	256	198	199	197	207
-2		269	271	271	264	201	202	200	208
-1		275	278	277	270	200	200	200	210
0		280	286	282	276	205	205	205	213
1		283	286	281	271	206	204	207	214
2		290	288	283	271	208	208	209	214
3		293	292	284	271	206	207	207	214
4		298	294	288	272	214	206	206	211
5		294	294	284	267	211	205	203	208
6		302	298	287	270	212	206	203	209
7		309	305	297	279	217	208	206	213
Bodyweight gain (g/animal)									
			*	**	**		*	*	*
Day 0-7		29	20	14	3	12	3	1	0
sd		6.0	5.0	7.4	4.9	2.7	8.8	5.7	8.0
% of control		-	69	50	11	-	25	10	-

sd Standard deviation

Williams' test: * $p \leq 0.05$, ** $p \leq 0.01$

TABLE 4

Food consumption - group mean values (g/animal)

Print No: 0019

Printed: 04-AUG-00

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
DAY								
-7	32	31	30	30	19	20	18	21
-6	31	32	32	30	19	20	20	22
-5	34	32	30	31	22	20	21	21
-4	29	29	30	30	20	20	22	22
-3	31	32	32	31	21	21	22	22
-2	31	32	32	30	18	18	20	22
-1	29	30	30	29	22	21	21	22
1	28	26	24	21	20	19	19	18
2	30	28	26	23	21	20	21	19
3	29	30	25	23	18	19	19	20
4	30	30	27	24	22	19	19	19
5	26	28	25	22	21	20	20	20
6	29	29	26	25	19	20	19	19
7	29	29	27	26	20	19	18	19
Cumulative (g/animal)								
Day 1-7	201	200	180	164	141	136	135	134
% of control	-	100	90	82	-	96	96	95

TABLE 5

Water consumption – group mean values (g/animal)

SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
DAY								
-7	32	32	32	29	22	24	22	27
-6	32	31	33	30	23	24	23	26
-5	31	33	34	32	23	24	24	27
-4	28	29	33	28	24	25	23	26
-3	32	31	32	30	24	25	24	26
-2	31	32	33	30	21	22	22	25
-1	32	33	33	32	29	29	27	28
1	30	29	29	25	23	20	23	26
2	34	32	34	29	25	27	27	30
3	31	34	32	31	20	26	27	28
4	31	32	35	31	30	25	25	27
5	28	31	30	27	25	26	27	27
6	30	27	27	25	22	24	22	25
7	29	28	28	27	22	23	21	24
Cumulative (g/animal)								
Day 1 – 7	214	214	215	194	169	172	171	186
% of control	-	100	100	91	-	102	102	110

TABLE 6

Cholinesterase activity – group mean values

Group		Plasm CHE U/L	RCHE DTNB U/L	Brain CHE U/Kg
1M				
	Mean	363	980	14020
	SD	47.0	77.9	483.0
	n	5	5	5
2M				
	Mean			
	SD			
	n			
3M				
	Mean			
	SD			
	n			
4M				
	Mean	364	970	14060
	SD	68.5	129.2	482.7
	n	5	5	5

TABLE 6

(Cholinesterase activity – continued)

Group		Plasm CHE U/L	RCHE DTNB U/L	Brain CHE U/Kg
1F				
	Mean	735	1135	13740
	SD	105.8	193.3	677.7
	n	5	5	5
2F				
	Mean			
	SD			
	n			
3F				
	Mean			
	SD			
	n			
4F				
	Mean	724	1090	13580
	SD	54.7	166.4	503.2
	n	5	5	5

TABLE 7

Macroscopic pathology – incidence summary

Print No: 0014

Printed: 25-SEP-00

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------	T-7499	-----
DOSAGE (PPM)	:	0	200	350	500

Xybion protocol number: MIN 251

		--- NUMBER-OF-ANIMALS-AFFECTED ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND KEYWORD(S) OR PHRASE	NUMBER:	5	5	5	5	5	5	5	5
** TOP OF LIST **									
LARYNX	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
LUNGS & BRONCHI	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
NASAL TURBINATES	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
TRACHEA	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
TRACHEAL BIFURC.	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
EYES	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
OPAQUE		2	0	0	0	0	0	0	0
HARDERIAN GLANDS	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
DARK		0	0	1	0	0	0	0	0
STOMACH	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
ANTRUM WHITE NODULE(S)		0	0	0	1	0	0	0	0
TEETH	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
UTERUS & CERVIX	NUMBER EXAMINED:	0	0	0	0	5	5	5	5
FLUID DISTENTION		0	0	0	0	0	3	2	3
** END OF LIST **									

TABLE 8

Organ weights - group mean values (g)

Print No: 0027

Printed: 04-AUG-00

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------	T-7499	-----
DOSAGE (PPM)	:	0	200	350	500

Xybion protocol number: MIN 251

	ABSOLUTE ORGAN WEIGHTS				BODYWEIGHTS ADJUSTED VALUES			
SEX:	MALE							
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	5	5	5	5				
TERMINAL BODY WEIGHT (g)								
				*				
N :	5	5	5	5				
MEAN :	304.7	303.4	296.1	276.4				
sd :	7.5	14.1	13.4	27.6				
ADRENALS					**ADRENALS**			
N :	5	5	5	5	N : 5	5	5	5
MEAN :	0.052	0.052	0.058	0.053	MEAN : 0.051	0.050	0.058	0.057
sd :	0.007	0.005	0.002	0.007				
EPIDIDYMIDES								
N :	5	5	5	5				
MEAN :	0.661	0.607	0.630	0.597				
sd :	0.064	0.127	0.055	0.051				
HEART					**HEART**			
N :	5	5	5	5	N : 5	5	5	5
MEAN :	1.253	1.206	1.092	1.092	MEAN : 1.209	1.168	1.088	1.178
sd :	0.096	0.101	0.142	0.187				
KIDNEYS					**KIDNEYS**			
								*
N :	5	5	5	5	N : 5	5	5	5
MEAN :	2.03	2.05	2.05	2.02	MEAN : 1.96	1.99	2.04	2.16
sd :	0.11	0.14	0.10	0.26				

Williams' test: * $p \leq 0.05$

TABLE 8

(Organ weights – continued)

Print No: 0027

Printed: 04-AUG-00

Xybion protocol number: MIN 251

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------	T-7499	-----
DOSAGE (PPM)	:	0	200	350	500

	ABSOLUTE ORGAN WEIGHTS				BODYWEIGHTS ADJUSTED VALUES				
SEX:	MALE								
GROUP:	1	2	3	4		1	2	3	4
NUMBER:	5	5	5	5					
LIVER					**LIVER**				
N	5	5	5	5	N	5	5	5	5
MEAN	13.22	12.47	11.67	11.95	MEAN	12.71	12.03	11.62	12.94
sd	1.47	0.81	0.80	1.80					
LUNGS & BRONCHI					**LUNGS AND BRONCHI**				
N	5	5	5	5	N	5	5	5	5
MEAN	1.140	1.185	1.132	1.086	MEAN	1.106	1.155	1.129	1.154
sd	0.031	0.084	0.041	0.119					
TESTES									
N	5	5	5	5					
MEAN	3.01	3.07	3.10	2.93					
sd	0.07	0.24	0.38	0.17					

No differences of statistical significance

TABLE 8

(Organ weights – continued)

Print No: 0028

Printed: 04-AUG-00

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------	T-7499	-----
DOSAGE (PPM)	:	0	200	350	500

Xybion protocol number: MIN 251

	ABSOLUTE ORGAN WEIGHTS				BODYWEIGHTS ADJUSTED VALUES			
SEX:	FEMALE							
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	5	5	5	5				
TERMINAL BODY WEIGHT (g)								
N	5	5	5	5				
MEAN	219.6	207.6	203.7	211.4				
sd	7.5	6.6	14.0	7.8				
ADRENALS								
N	5	5	5	5				
MEAN	0.067	0.063	0.069	0.071				
sd	0.009	0.005	0.008	0.015				
HEART					**HEART**			
N	5	5	5	5	5	5	5	5
MEAN	0.980	0.872	0.920	0.955	0.933	0.888	0.957	0.950
sd	0.115	0.069	0.155	0.044				
KIDNEYS								
N	5	5	5	5				
MEAN	1.57	1.48	1.49	1.57				
sd	0.13	0.08	0.08	0.13				
LIVER		**	**	**	**LIVER**			*
N	5	5	5	5	5	5	5	5
MEAN	9.60	7.99	8.08	8.56	9.18	8.13	8.39	8.52
sd	0.91	0.38	0.80	0.53				

Williams' test: * $p \leq 0.05$, ** $p \leq 0.01$

TABLE 8

(Organ weights – continued)

Print No: 0028

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Printed: 04-AUG-00

Xybion protocol number: MIN 251

	ABSOLUTE ORGAN WEIGHTS				BODYWEIGHTS ADJUSTED VALUES			
SEX:	FEMALE							
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	5	5	5	5				
LUNGS & BRONCHI								
N :	5	5	5	5				
MEAN :	1.004	0.914	0.983	0.941				
sd :	0.102	0.085	0.059	0.053				

No differences of statistical significance

TABLE 9

Microscopic pathology – expanded incidence summary

Print No: 0035

Printed: 21-AUG-00

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------ T-7499 -----		
DOSAGE (PPM)	:	0	200	350	500

Xybion prtocol number: MIN 251

		--- N U M B E R - O F - A N I M A L S - A F F E C T E D ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN/TISSUE EXAMINED	NUMBER:	5	5	5	5	5	5	5	5
** TOP OF LIST **									
LARYNX	NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--FOREIGN BODY REACTION - VENTRAL POUCH	Present>	0	0	0	0	1	0	0	0
	Total>	0	0	0	0	1	0	0	0
LUNGS & BRONCHI	NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--PNEUMONITIS	Minimal>	0	0	0	0	3	0	0	0
	Total>	0	0	0	0	3	0	0	0
--PNEUMONITIS WITH HAEMATOIDIN CRYSTALS	Minimal>	0	0	0	1	1	0	0	0
	Total>	0	0	0	1	1	0	0	0
--AGGREGATIONS OF ALVEOLAR MACROPHAGES	Minimal>	2	0	0	2	1	0	0	1
	Total>	2	0	0	2	1	0	0	1
--FOAMY ALVEOLAR MACROPHAGES	Minimal>	0	0	0	0	0	0	0	2
	Total>	0	0	0	0	0	0	0	2
--PERIVASCULAR EOSINOPHILS	Minimal>	0	0	0	0	2	0	0	1
	Total>	0	0	0	0	2	0	0	1
--ALVEOLAR OSSEOUS METAPLASIA	Minimal>	0	0	0	1	0	0	0	1
	Total>	0	0	0	1	0	0	0	1
NASAL TURBINATES	NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--EPITHELIAL INFLAMMATION-VENTRAL MEATUS	Minimal>	0	0	0	2	0	0	0	0
	Total>	0	0	0	2	0	0	0	0
--SUBEPITHELIAL INFLAMMATION-VENTRAL MEATUS	Minimal>	0	0	0	2	0	0	0	0
	Total>	0	0	0	2	0	0	0	0

TABLE 9

(Microscopic pathology – expanded incidence summary – continued)

Print No: 0035

Printed: 21-AUG-00

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion prtocol number: MIN 251

		--- NUMBER OF ANIMALS AFFECTED ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN/TISSUE EXAMINED	NUMBER:	5	5	5	5	5	5	5	5
** FROM PREVIOUS PAGE **									
NASAL TURBINATES	NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--CYSTIC DUCT OF BOWMAN'S GLAND	Minimal>	0	0	0	0	1	0	0	0
	Total>	0	0	0	0	1	0	0	0
--MINERALISATION-OLFACTORY EPITHELIUM	Minimal>	1	0	0	0	0	0	0	2
	Total>	1	0	0	0	0	0	0	2
TRACHEA	NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--SUBEPITHELIAL INFLAMMATION	Minimal>	0	0	0	1	0	0	0	0
	Total>	0	0	0	1	0	0	0	0
TRACHEAL BIFURC.	NUMBER EXAMINED:	5	0	0	4	5	0	0	5
EYES	NUMBER EXAMINED:	2	0	0	0	0	0	0	0
--ULCERATIVE KERATITIS WITH INFLAMMATION OF ANTERIOR SEGMEN	Present>	2	0	0	0	0	0	0	0
	Total>	2	0	0	0	0	0	0	0
HARDERIAN GLANDS	NUMBER EXAMINED:	0	0	1	0	0	0	0	0
--PROMINENT PORPHYRIN PIGMENT	Present>	0	0	1	0	0	0	0	0
	Total>	0	0	1	0	0	0	0	0
STOMACH	NUMBER EXAMINED:	0	0	0	1	0	0	0	0
--ECTOPIC NONGLANDULAR EPITHELIUM IN GLANDULAR MUCOSA, FOCA	Present>	0	0	0	1	0	0	0	0
	Total>	0	0	0	1	0	0	0	0
TEETH	NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--DISRUPTION OF AMELOBLAST LAYER	Minimal>	0	0	0	1	0	0	0	1
	Total>	0	0	0	1	0	0	0	1

TABLE 9

(Microscopic pathology – expanded incidence summary – continued)

Print No: 0035

Printed: 21-AUG-00

Xybion prtocol number: MIN 251

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------	T-7499	-----
DOSAGE (PPM)	:	0	200	350	500

		--- N U M B E R - O F - A N I M A L S - A F F E C T E D ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN/TISSUE EXAMINED	NUMBER:	5	5	5	5	5	5	5	5
UTERUS & CERVIX	NUMBER EXAMINED:	0	0	0	0	0	3	2	3
--LUMINAL DILATATION	Minimal>	0	0	0	0	0	0	0	1
	Slight>	0	0	0	0	0	1	0	1
	Moderate>	0	0	0	0	0	2	2	1
	Total>	0	0	0	0	0	3	2	3

** END OF LIST **

APPENDIX 1

Clinical signs post exposure – individual observations

Group	Animal number	Observations
1M (Control)	1 - 5	No abnormalities detected
2M (Low dose)	6	Noisy breathing (scratchy), Day 4
	7 - 10	No abnormalities detected
3M (Inter. dose)	11	No abnormalities detected
	12	Noisy breathing (scratchy), Day 2
	13	Sensitive to touch, Day 4. Noisy breathing (scratchy), Days 4 and 5
	14	Noisy breathing (scratchy), Day 5
	15	Sensitive to touch, Days 2 and 4. Noisy breathing (scratchy), Days 4 and 5
4M (High dose)	16	Slow and exaggerated breathing, Day 2. Noisy breathing (scratchy), Day 5
	17	Pilo-erection, Day 2
	18	Pilo-erection, Day 2. Slow and exaggerated breathing, Day 2. Noisy breathing (scratchy), Days 3 and 5
	19	Pilo-erection, Day 2. Slow and exaggerated breathing, Day 2. Noisy breathing (scratchy), Day 5
	20	Breathing rate slows followed by an audible 'pop', Day 1. Pilo-erection, Day 2. Slow and exaggerated breathing, Day 2. Noisy breathing (scratchy), Day 4
1F (Control)	21 - 25	No abnormalities detected
2F (Low dose)	26	Sensitive to touch, Day 5
	27 - 28	No abnormalities detected
	29	Sensitive to touch, Day 5
	30	No abnormalities detected
3F (Inter. dose)	31	Noisy breathing (scratchy), Days 2, 4 and 5. Sensitive to touch, Days 3 to 5
	32	Sensitive to touch, Days 2 to 5. Noisy breathing (scratchy), Day 2
	33	Noisy breathing (scratchy), Day 2
	34	Sensitive to touch, Days 2 to 5. Noisy breathing (scratchy), Day 5
	35	No abnormalities detected
4M (High dose)	36	Sensitive to touch, Day 2. Noisy breathing (scratchy), Days 2 to 4
	37	Sensitive to touch, Day 2. Noisy breathing (scratchy), Day 2. Slow and exaggerated breathing, Day 2
	38	Sensitive to touch, Days 2 and 3. Noisy breathing (scratchy), Days 2 and 3. Slow and exaggerated breathing, Day 2
	39	Sensitive to touch, Days 2 and 3. Slow and exaggerated breathing, Day 2. Noisy breathing (scratchy), Days 3 and 5
	40	Sensitive to touch, Days 2 to 4. Noisy breathing (scratchy), Days 2 and 3

APPENDIX 2

Daily clinical signs – individual observations

Print No: 0039

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Printed: 22-SEP-00

Xybion protocol number: MIN 251

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY / KEYWORD / QUALIFIER (GROUP: 1M)	DAYS 1-8
1	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
2	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
3	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
4	7	2	EYES	
			DRY	
			LEFT	7-8
			OPAQUE	
			LEFT	7-8
			PROMINENT	
			LEFT	5-6, 8
5	7	2	EYES	
			DRY	
			LEFT	7-8
			OPAQUE	
			LEFT	8
			PROMINENT	
			LEFT	5-8

APPENDIX 2

(Daily clinical signs – continued)

Print No: 0039

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Printed: 22-SEP-00

Xybion protocol number: MIN 251

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY KEYWORD QUALIFIER	GROUP: 2M DAYS 1-8
6	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
7	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
8	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
9	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
10	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	

APPENDIX 2

(Daily clinical signs – continued)

Print No: 0039

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Printed: 22-SEP-00

Xybion protocol number: MIN 251

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY KEYWORD QUALIFIER	GROUP: 3M DAYS 1-8
11	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
12	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
13	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
14	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
15	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	

APPENDIX 2

(Daily clinical signs – continued)

Print No: 0039

Printed: 22-SEP-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY KEYWORD QUALIFIER	GROUP: 4M DAYS 1-8
16	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
17	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
18	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
19	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
20	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	

APPENDIX 2

(Daily clinical signs – continued)

Print No: 0039

Printed: 22-SEP-00

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

CATEGORY GROUP: 1F

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	KEYWORD QUALIFIER	DAYS 1-8
21	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
22	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
23	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
24	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
25	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	

APPENDIX 2

(Daily clinical signs – continued)

Print No: 0039

Printed: 22-SEP-00

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

CATEGORY GROUP: 2F

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	KEYWORD QUALIFIER	DAYS 1-8
26	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
27	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
28	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
29	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
30	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	

APPENDIX 2

(Daily clinical signs – continued)

Print No: 0039

Printed: 22-SEP-00

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY KEYWORD QUALIFIER	GROUP: 3F DAYS 1-8
31	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
32	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
33	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
34	7	2	SKIN ABRASION DRY TAIL	3-8
35	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	

APPENDIX 2

(Daily clinical signs – continued)

Print No: 0039

Printed: 22-SEP-00

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY KEYWORD QUALIFIER	GROUP: 4F DAYS 1-8
36	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
37	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
38	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
39	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	
40	7	2	ANIMAL HAS NO SIGNIFICANT FINDINGS	

APPENDIX 3

Bodyweights - individual values (g)

Print No: 0022

Printed: 04-AUG-00

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------	T-7499	-----
DOSAGE (PPM)	:	0	200	350	500

Xybion protocol number: MIN 251

GROUP	ANIMAL	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
1M	1	281	285	288	293	296	297	299	306
	2	273	275	281	285	291	290	298	303
	3	283	289	298	299	303	301	309	317
	4	280	280	286	287	291	280	293	301
	5	284	286	298	302	307	303	309	319
2M	6	310	312	309	316	319	319	319	323
	7	269	271	271	281	280	278	283	293
	8	293	291	296	298	299	301	305	311
	9	290	287	288	292	294	293	299	307
	10	267	270	274	274	280	279	285	293
3M	11	279	277	278	280	283	278	280	292
	12	275	275	273	274	277	276	280	292
	13	287	287	288	289	296	292	295	304
	14	295	293	300	302	304	304	311	318
	15	274	272	273	275	277	268	272	277
4M	16	288	280	283	282	285	281	285	295
	17	297	293	294	291	292	289	292	299
	18	236	232	228	229	227	222	224	232
	19	288	283	283	282	281	276	283	295
	20	269	268	268	271	273	264	266	274

APPENDIX 3

(Bodyweights – continued)

Print No: 0023

Printed: 04-AUG-00

Xybion protocol number: MIN 251

GROUP		1	2	3	4
COMPOUND		CONTROL	------ T-7499 -----		
DOSAGE (PPM)		0	200	350	500

GROUP	ANIMAL	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
1F	21	198	199	200	201	205	199	200	211
	22	203	201	209	201	210	207	207	212
	23	205	208	210	211	213	211	215	222
	24	214	215	218	215	225	222	223	226
	25	206	208	205	205	219	217	215	216
2F	26	206	207	213	215	209	212	212	212
	27	207	201	205	203	201	199	202	201
	28	201	205	211	212	212	211	213	217
	29	209	209	208	206	210	204	202	205
	30	199	198	202	202	201	200	201	203
3F	31	219	219	223	220	220	218	219	224
	32	192	197	202	201	195	195	196	197
	33	215	216	213	214	216	212	210	218
	34	203	201	202	199	201	197	193	195
	35	198	202	204	202	199	193	197	198
4F	36	212	207	205	207	205	203	201	204
	37	211	208	210	206	206	206	208	211
	38	207	215	215	215	212	210	215	219
	39	217	216	215	215	215	208	205	211
	40	217	226	225	226	219	216	216	220

APPENDIX 4

Cholinesterase activity – individual values

Group	Animal	Plasm CHE U/L	RCHE DTNB U/L	Brain CHE U/Kg
1M	1	375	1050	14200H
	2	439	950	14100H
	3	327	925	14650H
	4	347	900	13800H
	5	326	1075	13350H
2M	6			
	7			
	8			
	9			
	10			
3M	11			
	12			
	13			
	14			
	15			
4M	16	410	1100	14500H
	17	345	950	13900H
	18	270	1075	13300H
	19	348	950	14400H
	20	449	775	14200H

APPENDIX 4

(Cholinesterase activity – continued)

Group	Animal	Plasm CHE U/L	RCHE DTNB U/L	Brain CHE U/Kg
1F	21	810	1050	13900H
	22	639	1475	12900H
	23	602	1050	14750H
	24	802	1000	13700H
	25	823	1100	13450H
2F	26			
	27			
	28			
	29			
	30			
3F	31			
	32			
	33			
	34			
	35			
4F	36	702	1300	14450H
	37	738	1025	13550H
	38	668	900	13300H
	39	811	1225	13200H
	40	700	1000	13400H

APPENDIX 5

Absolute organ weights - individual values (g)

Print No: 0029

Printed: 04-AUG-00

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

GROUP	ANIMAL	TERMINAL BODY WT (g)	ADRENALS	EPIDIDYMID	HEART	KIDNEYS	LIVER	LUNGS & BR	TESTES	LEFT BRAIN
1M	1	303.6	0.059	0.757	1.205	2.19	12.64	1.150	3.11	0.851
	2	298.7	0.042	0.679	1.118	2.05	11.25	1.137	3.03	0.850
	3	310.8	0.060	0.593	1.254	2.06	12.99	1.156	3.02	0.902
	4	296.6	0.052	0.614	1.343	1.95	15.11	1.088	2.93	0.905
	5	313.8	0.049	0.664	1.344	1.90	14.10	1.169	2.97	0.992
2M	6	323.4	0.054	0.627	1.128	2.20	13.52	1.267	3.38	0.917
	7	292.0	0.054	0.639	1.194	2.07	12.21	1.142	3.03	0.988
	8	310.4	0.052	0.721	1.332	2.17	13.11	1.276	2.98	0.939
	9	302.5	0.056	0.659	1.283	1.91	11.66	1.081	3.22	1.014
	10	288.7	0.043	0.390	1.092	1.92	11.84	1.159	2.75	0.964
3M	11	292.2	0.055	0.582	1.078	2.05	11.75	1.090	2.73	0.934
	12	290.6	0.061	0.681	1.264	1.97	10.83	1.199	3.73	0.995
	13	302.5	0.057	0.699	1.169	2.03	12.47	1.133	3.15	0.954
	14	315.3	0.059	0.602	1.068	2.21	12.41	1.129	2.95	0.946
	15	280.0	0.060	0.587	0.882	1.98	10.88	1.109	2.96	0.964
4M	16	291.4	0.054	0.529	1.243	2.02	12.61	1.153	3.03	0.885
	17	296.3	0.061	0.647	1.039	2.26	13.16	1.171	3.05	0.901
	18	229.9	0.043	0.648	0.799	1.65	9.73	0.893	2.75	0.754
	19	292.1	0.059	0.591	1.258	2.26	13.86	1.166	3.07	0.734
	20	272.4	0.049	0.568	1.120	1.92	10.38	1.049	2.74	0.819

APPENDIX 5

(Absolute organ weights – continued)

Print No: 0030

Printed: 04-AUG-00

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

GROUP	ANIMAL	TERMINAL BODY WT (g)	ADRENALS	HEART	KIDNEYS	LIVER	LUNGS & BR	LEFT BRAIN
1F	21	210.0	0.055	0.955	1.42	9.24	0.977	0.843
	22	214.3	0.071	1.102	1.55	8.69	0.898	0.877
	23	222.9	0.070	0.857	1.47	9.69	0.938	0.937
	24	228.9	0.061	1.098	1.75	11.09	1.156	0.954
	25	222.0	0.079	0.888	1.67	9.27	1.052	0.862
2F	26	213.0	0.066	0.868	1.49	8.19	0.937	0.753
	27	200.9	0.064	0.893	1.56	7.79	1.020	0.930
	28	215.9	0.063	0.942	1.55	8.56	0.950	0.913
	29	206.2	0.054	0.901	1.39	7.62	0.867	0.860
	30	202.1	0.066	0.758	1.39	7.80	0.798	0.856
3F	31	221.2	0.071	1.153	1.58	8.60	0.998	1.072
	32	193.2	0.061	0.722	1.56	8.30	0.916	0.917
	33	216.2	0.077	0.940	1.42	8.94	0.926	1.015
	34	190.6	0.076	0.919	1.47	7.58	1.040	0.982
	35	197.1	0.060	0.868	1.42	6.97	1.037	0.933
4F	36	200.0	0.080	0.971	1.64	8.90	0.871	0.821
	37	208.2	0.072	0.918	1.51	7.69	0.927	0.856
	38	217.2	0.090	0.985	1.71	8.72	0.919	0.889
	39	211.9	0.059	1.000	1.37	8.44	0.998	0.818
	40	219.8	0.054	0.899	1.63	9.05	0.991	0.944

APPENDIX 6

Individual pathological findings

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL      ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0001           SEX: MALE           DOSE GROUP: 1       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   303.6 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Printed: 21-AUG-00

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0002 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 298.7 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	NASAL TURBINATES : -MINERALISATION-OLFACTORY EPITHELIUM,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Printed: 21-AUG-00

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0003 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 310.8 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI :
	-AGGREGATIONS OF ALVEOLAR MACROPHAGES,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0004 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 296.6 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
EYES : -OPAQUE, MINIMAL; LEFT.	EYES : -ULCERATIVE KERATITIS WITH INFLAMMATION OF ANTERIOR SEGMENT,-PRESENT >NOTE:>LESION UNILATERAL LUNGS & BRONCHI : -AGGREGATIONS OF ALVEOLAR MACROPHAGES,-MINIMAL, FOCAL TRACHEAL BIFURC. : >NOTE:>POINT OF BIFURCATION NOT IN SECTION EXAMINED

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :    CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0      200      350      500
```

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0005 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 313.8 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
EYES : -OPAQUE, MODERATE; LEFT.	EYES : -ULCERATIVE KERATITIS WITH INFLAMMATION OF ANTERIOR SEGMENT,-PRESENT >NOTE:>LESION UNILATERAL

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0006           SEX: MALE           DOSE GROUP: 2       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   323.4 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0007           SEX: MALE           DOSE GROUP: 2       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   292.0 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL      ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0008           SEX: MALE           DOSE GROUP: 2       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   310.4 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0009           SEX: MALE           DOSE GROUP: 2       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   302.5 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0010           SEX: MALE           DOSE GROUP: 2       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   288.7 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0011           SEX: MALE           DOSE GROUP: 3       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   292.2 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0012           SEX: MALE           DOSE GROUP: 3       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   290.6 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4 Printed: 21-AUG-00
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0013 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 302.5 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
ANIMAL NUMBER: 0014          SEX: MALE          DOSE GROUP: 3       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00     STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   315.3 GRAMS
```

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
HARDERIAN GLANDS :	HARDERIAN GLANDS :
-DARK, MODERATE; LEFT.	-PROMINENT PORPHYRIN PIGMENT,-PRESENT

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0015           SEX: MALE           DOSE GROUP: 3       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   280.0 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
ANIMAL NUMBER: 0016          SEX: MALE          DOSE GROUP: 4       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00     STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   291.4 GRAMS
```

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	NASAL TURBINATES : -EPITHELIAL INFLAMMATION-VENTRAL MEATUS,-MINIMAL, FOCAL -SUBEPITHELIAL INFLAMMATION-VENTRAL MEATUS,-MINIMAL, FOCAL TEETH : -DISRUPTION OF AMELOBLAST LAYER,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------ T-7499 -----		
DOSAGE (PPM)	:	0	200	350	500

Xybion protocol number: MIN 251

: 70 :

ANIMAL NUMBER: 0017 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE

DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 296.3 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI : -ALVEOLAR OSSEOUS METAPLASIA,-MINIMAL
	TRACHEA : -SUBEPITHELIAL INFLAMMATION,-MINIMAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 251/003341

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4 Printed: 21-AUG-00
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0018 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 229.9 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
STOMACH : -ANTRUM WHITE NODULE(S); MUCOSA, ONE, NEAR TO LIMITING RIDGE, 1MM.	STOMACH : -ECTOPIC NONGLANDULAR EPITHELIUM IN GLANDULAR MUCOSA, FOCAL,-PRESENT TRACHEAL BIFURC. : >TISSUE MISSING

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4 Printed: 21-AUG-00
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0019 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 292.1 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI :
	-AGGREGATIONS OF ALVEOLAR MACROPHAGES,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
ANIMAL NUMBER: 0020           SEX: MALE           DOSE GROUP: 4       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   272.4 GRAMS
```

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI : -PNEUMONITIS WITH HAEMATOIDIN CRYSTALS,-MINIMAL, FOCAL -AGGREGATIONS OF ALVEOLAR MACROPHAGES,-MINIMAL, FOCAL
	NASAL TURBINATES : -EPITHELIAL INFLAMMATION-VENTRAL MEATUS,-MINIMAL, FOCAL -SUBEPITHELIAL INFLAMMATION-VENTRAL MEATUS,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
ANIMAL NUMBER: 0021          SEX: FEMALE         DOSE GROUP: 1       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00     STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   210.0 GRAMS
```

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LARYNX : -FOREIGN BODY REACTION - VENTRAL POUCH,-PRESENT LUNGS & BRONCHI : -PNEUMONITIS,-MINIMAL, FOCAL -PNEUMONITIS WITH HAEMATOIDIN CRYSTALS,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4 Printed: 21-AUG-00
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0022 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 214.3 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI : -PERIVASCULAR EOSINOPHILS,-MINIMAL
	NASAL TURBINATES : -CYSTIC DUCT OF BOWMAN'S GLAND,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0023           SEX: FEMALE          DOSE GROUP: 1       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   222.9 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Printed: 21-AUG-00

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0024 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 228.9 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI :
	-PNEUMONITIS,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4 Printed: 21-AUG-00
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0025 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 222.0 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI : -PNEUMONITIS,-MINIMAL, FOCAL -AGGREGATIONS OF ALVEOLAR MACROPHAGES,-MINIMAL, FOCAL -PERIVASCULAR EOSINOPHILS,-MINIMAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0026 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 213.0 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
UTERUS & CERVIX :	UTERUS & CERVIX :
-FLUID DISTENTION, MINIMAL	-LUMINAL DILATATION,-MODERATE

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0027 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 200.9 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
UTERUS & CERVIX :	UTERUS & CERVIX :
-FLUID DISTENTION, MODERATE	-LUMINAL DILATATION,-MODERATE

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0028 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 215.9 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
UTERUS & CERVIX :	UTERUS & CERVIX :
-FLUID DISTENTION, MINIMAL	-LUMINAL DILATATION,-SLIGHT

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0029           SEX: FEMALE          DOSE GROUP: 2       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   206.2 GRAMS
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    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL      ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0030           SEX: FEMALE          DOSE GROUP: 2       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   202.1 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
-------------------------------------------------------------------------------------------------------------------------------

    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

-------------------------------------------------------------------------------------------------------------------------------
```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0031 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 221.2 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
UTERUS & CERVIX :	UTERUS & CERVIX :
-FLUID DISTENTION, MINIMAL	-LUMINAL DILATATION,-MODERATE

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------	T-7499	-----
DOSAGE (PPM)	:	0	200	350	500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0032 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 193.2 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
UTERUS & CERVIX :	UTERUS & CERVIX :
-FLUID DISTENTION, MODERATE	-LUMINAL DILATATION,-MODERATE

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4 Printed: 21-AUG-00
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0033 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 216.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0034           SEX: FEMALE          DOSE GROUP: 3       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   190.6 GRAMS
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    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

```
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0035           SEX: FEMALE          DOSE GROUP: 3       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   197.1 GRAMS
-------------------------------------------------------------------------------------------------------------------------------
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    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

    *** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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```

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4 Printed: 21-AUG-00
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0036 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 200.0 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI : -ALVEOLAR OSSEOUS METAPLASIA,-MINIMAL
	NASAL TURBINATES : -MINERALISATION-OLFACTORY EPITHELIUM,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

GROUP : 1 2 3 4 Printed: 21-AUG-00
COMPOUND : CONTROL ------ T-7499 -----
DOSAGE (PPM) : 0 200 350 500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0037 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 208.2 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	NASAL TURBINATES : -MINERALISATION-OLFACTORY EPITHELIUM,-MINIMAL, FOCAL
UTERUS & CERVIX : -FLUID DISTENTION, MODERATE	UTERUS & CERVIX : -LUMINAL DILATATION,-MODERATE

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------	T-7499	-----
DOSAGE (PPM)	:	0	200	350	500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0038 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 217.2 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI : -AGGREGATIONS OF ALVEOLAR MACROPHAGES,-MINIMAL, FOCAL
	TEETH : -DISRUPTION OF AMELOBLAST LAYER,-MINIMAL, FOCAL
UTERUS & CERVIX : -FLUID DISTENTION, MODERATE	UTERUS & CERVIX : -LUMINAL DILATATION,-SLIGHT

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

```
GROUP              :        1        2        3        4
COMPOUND           :     CONTROL     ------ T-7499 -----
DOSAGE (PPM)       :        0       200      350      500
```

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0039 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 211.9 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI :
	-FOAMY ALVEOLAR MACROPHAGES,-MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

(Individual pathological findings – continued)

Print No: 0034

Printed: 21-AUG-00

GROUP	:	1	2	3	4
COMPOUND	:	CONTROL	------	T-7499	-----
DOSAGE (PPM)	:	0	200	350	500

Xybion protocol number: MIN 251

ANIMAL NUMBER: 0040 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 17-JUL-00 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 219.8 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI : -FOAMY ALVEOLAR MACROPHAGES,-MINIMAL, FOCAL -PERIVASCULAR EOSINOPHILS,-MINIMAL
UTERUS & CERVIX : -FLUID DISTENTION, MODERATE	UTERUS & CERVIX : -LUMINAL DILATATION,-MINIMAL

ADMINISTRATION OF T-7499

BY INHALATION TO RATS

Author

Ian Gilkison

CONTENTS **Page**

TEST SUBSTANCE AND ADMINISTRATION

TEST SUBSTANCE

The test substance, which is referred to throughout this report as T-7499, is 96 – 98% perfluorobutane sulfonyl fluoride (PBSF) and 2 – 4% perfluorosulfolane. It was supplied as a liquid in a small cylinder (net contents 35 kg/cylinder) and was received at these laboratories on 26 April 2000. Information from the Sponsor indicated that T-7499 was sufficiently stable for use in this study. The T-7499 was administered to the rats as a vapour diluted with air.

ADMINISTRATION

The T-7499 vapour was administered to the rats by whole-body exposure in chambers described below. The chamber atmospheres were produced by metering the liquid test substance into sintered glass vaporisers, through which flowed a stream of dried air. The atmosphere produced by the generation system was further diluted with air to give the final chamber concentrations of test aerosol.

The flow of air to the vapour generation systems and the diluent air supply were calibrated using a dry type gas meter during the preliminary phase of the study. Airflow through the generation systems was monitored throughout each of the exposures using in-line tapered tube gas flowmeters.

The settings of the test substance metering system required to obtain the target chamber concentrations were determined during the preliminary generation trials without animals, based on the Gas Chromatographic analysis of atmosphere samples. Minor adjustments were made to the test material delivery rates in order to maintain chamber concentrations close to target.

Animals assigned to the control group received an exposure to compressed air only, from the same source as used for the generation of the test atmospheres. All animals were exposed for 6 hours daily for five consecutive days.

The usage of T-7499 was determined, for each day of treatment, for each of the three test groups.

EXPOSURE SYSTEM

Each exposure system comprised a whole body inhalation exposure chamber, a vapour generator, a test material syringe pump, diluent control valves, a compressed air supply and control valves to each generator and in-line airflow monitoring flowmeters.

Schematic diagrams of the vapour generation system and an exposure chamber system are presented in Figures A and B. The component parts of the systems are described in further detail overleaf:

Vapour generation

The vapour was generated by metering the test substance from a polypropylene syringe (Fortuna) located on a syringe driver (Precidor, Model 5003, Infors HT, Switzerland). The syringes were cooled by a surrounding coil of copper tubing through which iced water was circulated. The liquid test material was delivered to the inlet of a glass vaporiser (Figure A) *via* a PolyTetraFluoroEthylene (PTFE) needle. Air was passed through the glass vessel vaporiser at a rate of 50 l/min. The vapour/air mixture passed out of the vaporiser into the chamber inlet ducting. Different chamber concentrations were achieved by varying the syringe pump infusion rate.

A compressor supplied the generation and dilution air to the vapour generators. The air was filtered to remove any residual particulate and was dried (dew point ~2°C).

For all groups the vapour/air mixture produced passed along the chamber inlet ducting to a tangential inlet mounted at the apex of the chamber where it was mixed with additional diluent air at a rate (approximately 100 l/min) sufficient to maintain the total chamber airflow at approximately 150 l/min.

The control group received clean air only at a rate of approximately 150 l/min.

Inhalation chamber

The exposure chambers were of stainless steel and glass construction and consisted of a cuboidal body fitted with a pyramidal base and top. The internal volume of each chamber was approximately $0.75m^3$. At the apex of the upper pyramidal figure was the tangentially mounted air duct. Immediately below this was a perforated canister, which ensured equal distribution of the test atmosphere within the chamber.

Access to the chamber was through the front of the box section *via* a hinged door with a glass panel and stainless steel frame. The door was sealed using moulded rubber sealing strip.

Exposure cages constructed of stainless steel mesh were suspended on a framework arranged on 4 levels. Each level held four cages, with each cage capable of housing 4 rats individually. This gave a total animal exposure capacity of 64 rats. In this investigation, 10 animal compartments were used on Level 2 and air samples were withdrawn for analysis from this level. No cages were present on Levels 1 or 4.

A wet and dry alcohol bulb thermohygrometer was suspended in the chamber. This was visible through the glass panelled door and was used to monitor chamber temperature and relative humidity.

The pyramidal base of each chamber was fitted with a 2-inch drain. The drain connected with a common drainage system *via* a ball valve.

A square tubular exhaust plenum, 3 inches in diameter and perforated along the ventral surface, was situated in the pyramidal base. This connected to the main extract system.

The total chamber airflow was 150 litres/minute. Air entered the chamber through the inlet duct. Diluent air flow was measured using a tapered tube flow meter situated at the front of a purpose-built stainless steel trolley on which the elutriator was mounted. Generation air was measured on a similar flowmeter mounted on the vapour generation trolley.

A Magnehelic pressure gauge (0-100mm water gauge) was connected with each chamber by a nylon tube. This was mounted on the elutriator trolley and was used to monitor the atmosphere pressure inside the chamber, relative to the exposure room.

Extraction of the chambers was accomplished by means of a single fan mounted on the outside wall of the building withdrawing air through a manifold to which all chambers were connected. The chamber air extract was vented to atmosphere *via* an exhaust stack.

Extract flow was adjusted using gate valves mounted in the extract ducting between the chamber and filters. The internal pressure within each chamber was maintained at approximately –5 mm water pressure below ambient when operational.

The control animals were exposed using a similar system to that used for the test groups, but received compressed air only.

PROCEDURE

A separate exposure chamber was used for each group. The Control animals were exposed using an identical exposure chamber to that used for the test groups. The inhalation system was set up as described above.

A polypropylene syringe (50 ml) was loaded with test material and the net volume and gross weight recorded prior to the exposure. The syringe was attached to the infusion pump and connected to the delivery line (PTFE tubing), the outlet of which was positioned close to the glass frit of the vaporiser. The test substance was advanced to the end of the delivery line ready for the start of the exposure.

The rats were removed from their cages and placed into the individual compartments of the exposure cages from the rear left hand side to front right hand side. The animals were located on Level 2 of the chamber. In order to avoid any variations in the dose received due to the spatial arrangements of the animals within the chamber, the position of the animals within the chamber was changed randomly on a daily basis with males on the left and females on the right at the front of the chamber.

The chamber doors were closed and secured and the diluent and generator airflows were switched on. The chamber sampling ports were sealed and the Magnehelic gauges checked to ensure that operation of the chamber took place with internal pressure below that of the room.

The inhalation system was set up as described previously; the air supply and extract lines were calibrated and attached to the elutriator and chamber respectively. The exposure was started by turning on the infusion pump at the designated setting (presented in Table A). Adjustment of the setting was made at the discretion of the Study Director in order to maintain the vapour concentration close to target.

As required during the exposure, when all the test substance in the syringe had been delivered to the vaporiser, another loaded pre-weighed syringe was attached to the infusion pump. This was typically performed once during the Group 2 exposures; twice during the Group 3 exposures and thrice during the Group 4 exposures.

At intervals of 30 minutes, the operating parameters were noted and the condition of animals in the chambers was visually appraised. Generation checks and temperature were also recorded at 30-minute intervals throughout each exposure.

Samples of air for the determination of the chamber concentration of T-7499 were taken on at least six occasions from Level 2 of the chamber, at approximately hourly intervals. Additional samples were obtained as appropriate following adjustment of the generation system

At the end of the 6-hour exposure period the infusion pump was turned off. The syringe was removed, the net volume and final gross weight recorded, in order to calculate the nominal chamber concentration based on the total test substance used during the exposure. The generator and diluent airflows were turned off and the chambers allowed to clear. Clearance air was allowed to enter each chamber through an open sampling port in the chamber wall. At the end of the clearance period, the rats were unloaded from the chambers into their respective holding cages. The chambers were then washed to remove animal waste.

A summary of the operating conditions used is presented in Table A.

TARGET CONCENTRATIONS

The target concentrations of T-7499 were as follows:

Group	Designation	Concentration (ppm)
2	(Low dose)	200
3	(Inter. dose)	350
4	(High dose)	500

The target concentrations were selected in consultation with the Sponsor, following the review of available data.

EXPOSURE CHAMBER CONDITIONS

Analysis of chamber concentrations of T-7499

During the first exposure only, samples of chamber air were collected in gas tight syringes and injected directly into the sample loop of the gas chromatograph. Subsequent exposures were monitored using an automated system for on-line sampling and injection. The air samples were collected in sequence from Groups 4 to 2. Methods of sample collection and analysis are described in the Appendix below.

The method of analysis was adapted from a method supplied by the Sponsor to accommodate the Inhalation Toxicology Department equipment and procedures. Details of the analytical processes used are given in Appendix A.

Airflow

The air flows into and extracted from each chamber were monitored continuously using tapered tube rotameters and recorded at approximately 30-minute intervals throughout each exposure.

Temperature and relative humidity

The temperature of the generation water bath and also the air in each exposure chamber was recorded at approximately 30-minute intervals during each exposure.

The wet and dry bulb temperatures of a thermohygrometer placed in each chamber were recorded at 30-minute intervals throughout each exposure. Relative humidity was found using a look-up table supplied with the instrument.

CALCULATIONS

In order to minimise the cumulative errors, which result from repeated rounding of numbers, much of the data in this report has been calculated continuously using unrounded numbers and only rounded for printing. Consequently, any further calculations using these rounded numbers will include rounding errors in the last significant figure, possibly leading to small apparent discrepancies with other data in the report.

RESULTS

VAPOUR CONCENTRATION

Analysed concentration of T-7499

Analysed concentrations were in good agreement with target concentrations. The data are presented as follows:

Table B Daily mean concentrations
Appendix B Individual sample values

The coefficients of variation of the daily means were 2.0% 11.1% and 4.7 % for Groups 2, 3 and 4 respectively. The target and study mean concentrations of T-7499 are presented below:

Group	Concentration (ppm)	
	Target	Analysed
2 (Low dose)	200	203
3 (Inter dose)	350	345
4 (High dose)	500	507

Nominal concentration of T-7499

The data are presented in Table C and are summarised below:

Group	Study mean nominal concentration (ppm)	A/N (%)
2 (Low dose)	203	101.4
3 (Inter. dose)	345	87.0
4 (High dose)	507	99.5

$$A / N = \left(\frac{\text{Analysed concentration}}{\text{Nominal concentration}} \right) \times 100$$

The nominal concentrations were calculated using the mass of T-7499 vaporised during the exposures together with the chamber temperature, the molar gas constant and molecular weight of PBSF. The generation efficiency of the Group 3 system was slightly below the typical figure for this type of system (approximately 95%). This may be attributed to a number of factors including uncertainty in the various measurements and incomplete sealing of the exposure chamber doors.

Individual exposure measurements of the nominal concentration showed general agreement for each test group in comparison with the analysed values with A/N ratios in the ranges 99 - 106%, 74 – 95% and 95 - 105% for Groups 2, 3 and 4 respectively.

CHAMBER TEMPERATURE AND RELATIVE HUMIDITY

The daily mean chamber temperatures and relative humidities are presented in Table D. The chamber temperatures were similar for all groups throughout the duration of the study.

The study mean relative humidity (RH) for Group 1 (Control) was 36% and that of the test groups was 30 ±3%. These observed differences in chamber humidity had no discernible effect upon the animals and are considered not to have affected the outcome of the study.[§]

DISCUSSION

Control of the T-7499 vapour generation was satisfactory and was reflected in the low coefficients of variation for the daily mean concentrations (2.0%, 11.1% and 4.7% respectively for Groups 2, 3 and 4).

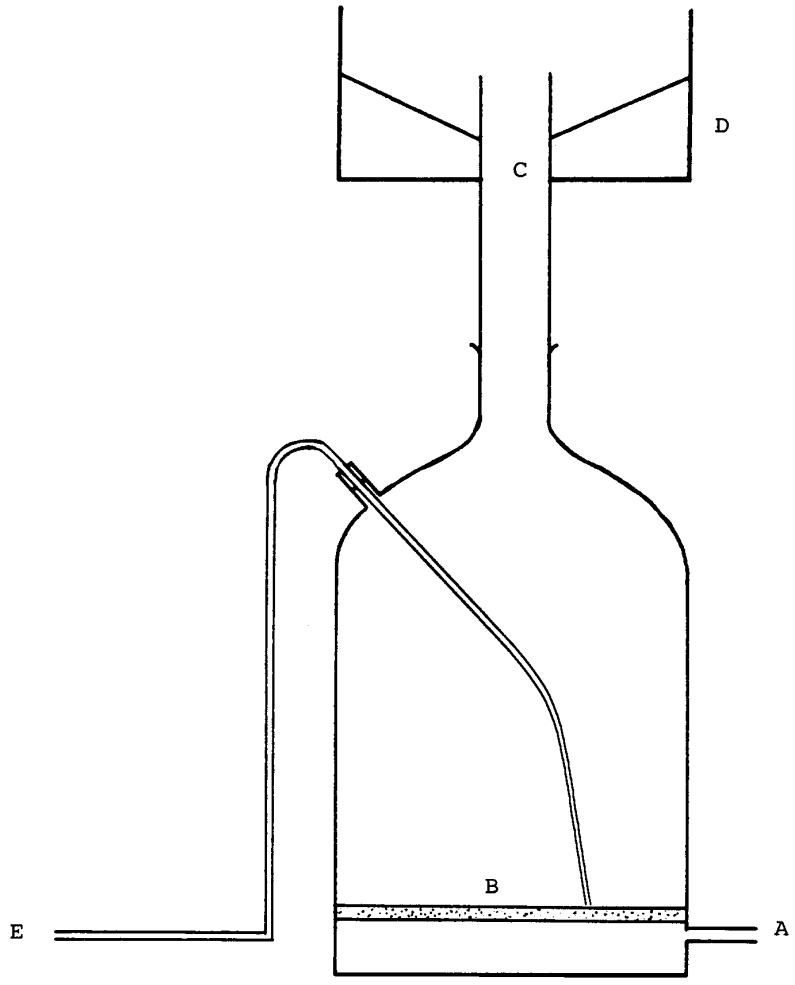
The study mean concentrations of T-7499 in each chamber were within 2% of the target concentrations for all Groups. There was typically less than 5% variation in the sample values across the six-hour exposures and this is considered acceptable within the experimental design and methods of generation and analysis used.

Good agreement was also observed between the analysed and the nominal chamber concentration values for Groups 2 and 4 (Low and high doses respectively) where study mean analysed/nominal (A/N) ratios of 99% and 101% were observed. For Group 3 (Intermediate dose) the A/N ratio was slightly low at 87%, possibly due to ingress of air through the chamber seals.

[§] Pauluhn, J and Mohr, U. (1999) Repeated 4-week Inhalation exposure of rats: effect of low, intermediate and high-humidity chamber atmospheres. *Exp. Toxic. Pathol. ,* **51,** 178-187.

FIGURE A

Schematic diagram of the vapour generation system



Key

A. Air line

B. Fritted glass disc

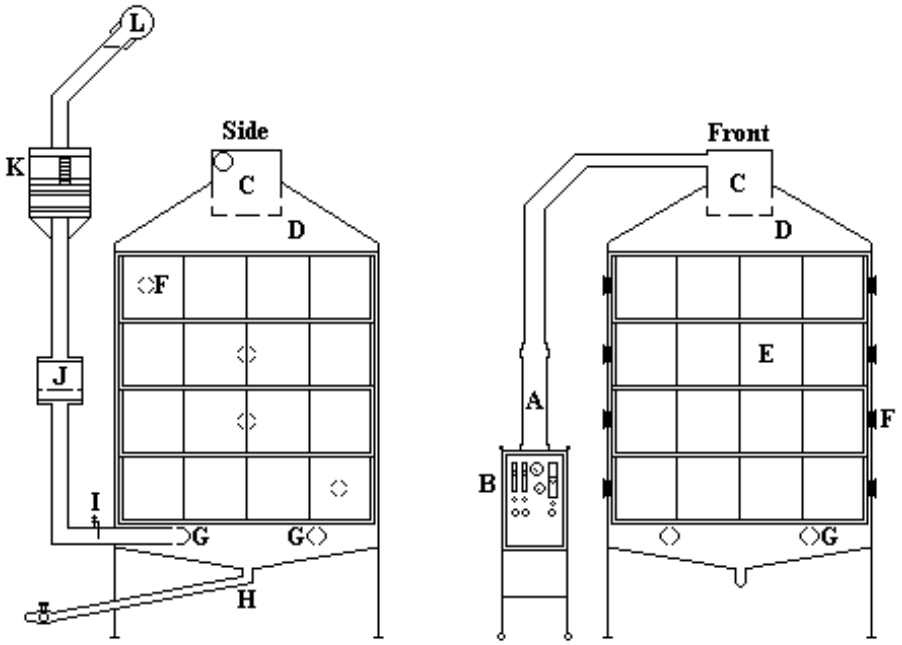
C. Vapour outlet

D. Stainless steel and glass elutriator

E. Test substance supply line

FIGURE B

Schematic of the exposure system used to expose rats



Key

A Glass elutriator	G Exhaust plenum
B Air flow control and chamber monitoring	H Drain
C Dispersion device	I Gate valve
D Exposure chamber (0.75 m^3)	J Pre-filter
E Animal exposure cages	K Powered extract filter
F Sampling port	L Main exhaust

TABLE A

Operating conditions of the exposure system

	Group			
	1 (Control)	2 (Low dose)	3 (Inter. dose)	4 (High dose)
Target concentration (ppm)	0	200	350	500
Atmosphere generation				
Test material feed	N/A	Precidor 5003 syringe pump		
Syringe size (ml)	N/A	50	50	50
Syringe pump setting (Speed, mm/min):				
Exposure 1	N/A	0.30	0.63	0.84
Exposure 2	N/A	0.33[a]; 0.34[b]	0.69[c]	0.87[d]
Exposure 3	N/A	0.33[e]	0.67[f]	0.87
Exposure 4	N/A	0.33	0.63	0.87
Exposure 5	N/A	0.33	0.60	0.84
Chamber airflows (l/min)				
Elutriator output (chamber input)	N/A	50	50	50
Chamber extract	150	150	150	150

[a] Syringe pump changed from previous setting after 108 minutes of the exposure.
[b] Syringe pump changed from previous setting after 196 minutes of the exposure.
[c] Syringe pump changed from previous setting after 199 minutes of the exposure.
[d] Syringe pump changed from previous setting after 200 minutes of the exposure.
[e] Syringe pump changed from previous setting after 142 minutes of the exposure.
[f] Syringe pump changed from previous setting after 127 minutes of the exposure.

TABLE B

Chamber concentrations of T-7499 – daily mean values

Exposure No.	Chamber concentration (ppm)		
	Group 2 (Low dose)	Group 3 (Intermediate dose)	Group 4 (High dose)
1	200	360	494
2	198	280	493
3	209	368	503
4	204	374	549
5	203	344	495
Mean	203	345	507
sd	4.2	38.2	24.1
CV (%)	2.0	11.1	4.7

sd standard deviation
CV Coefficient of Variation = (sd/mean) x 100

TABLE C

Nominal concentrations of T-7499

Group 2 (Low dose) : Target concentration 200 ppm

Exposure	Wt used (g)	Volume as vapour[1] (l)	Concentration (ppm)		A/N
			Nominal[2]	Analysed	
1	127.5	10.2	189	200	106.0
2	134.3	10.7	199	198	99.7
3	140.2	11.2	207	209	100.8
4	137.1	11.0	203	204	100.6
5	137.4	11.0	203	203	99.9
Mean	135.3	10.8	200	203	101.4
sd	4.83	0.39	7.2	4.2	2.64

Group 3 (Intermediate dose): Target concentration 350 ppm

Exposure	Wt used (g)	Volume as vapour[1] (l)	Concentration (ppm)		A/N
			Nominal[2]	Analysed	
1	257.9	20.6	381	360	94.4
2	255.3	20.4	378	280	74.2
3	284.1	22.7	420	368	87.6
4[3]				374	
5	253.4	20.2	375	344	91.8
Mean	262.7	21.0	388	345	87.0
sd	14.40	1.15	21.3	38.1	9.00

sd Standard deviation

A/N Analysed/nominal concentration ratio expressed as a percentage

[1] Calculated from the following equation:

$$V = \frac{W \times T \times R}{Mw}$$

where W = Weight of test material used in the exposure (g)
T = Chamber temperature (K)
R = Gas constant (0.08205 L Atm mol^{-1} K^{-1})
Mw = Molecular weight of PBSF (302.09 g/mol)

[2] Calculated from the following equation

$$\text{Nominal concentration} = \frac{V}{Va+V} \times 10^6$$

where Va = Chamber airflow (l) for the exposure (54000 litres)

[3] Nominal concentration not calculateable as total weight loss not measured.

TABLE C

(Nominal concentrations of T-7499 – continued)

Group 4 (High dose) : Target concentration 500 ppm

Exposure	Wt used (g)	Volume as vapour[1] (l)	Concentration (ppm)		A/N
			Nominal[2]	Analysed	
1	340.7	27.2	504	494	98.1
2	335.9	26.8	497	493	99.3
3	355.8	28.4	526	503	95.6
4	355.8	28.4	526	549	104.3
5	334.2	26.7	494	495	100.2
Mean	344.5	27.5	509	507	99.5
sd	10.61	0.85	15.7	23.9	3.21

sd Standard deviation

A/N Analysed/nominal concentration ratio expressed as a percentage

[1] Calculated from the following equation:

$$V = \frac{W \times T \times R}{Mw}$$

where W = Weight of test material used in the exposure (g)
T = Chamber temperature (K)
R = Gas constant (0.08205 L Atm mol^{-1} K^{-1})
Mw = Molecular weight of PBSF (302.09 g/mol)

[2] Calculated from the following equation

$$\text{Nominal concentration} = \frac{V}{Va+V} \times 10^6$$

where Va = Chamber airflow (l) for the exposure (54000 litres)

TABLE D

Chamber temperature and relative humidity – exposure mean values

Exposure	Mean chamber temperatures (°C) and relative humidity (%RH)							
	Group 1 (Air control)		Group 2 (Low dose)		Group 3 (Intermediate dose)		Group 4 (High dose)	
	Temp	RH	Temp	RH	Temp	RH	Temp	RH
1	21	35	21	26	20	33	21	29
2	21	35	21	27	21	32	21	30
3	21	39	21	29	21	33	22	32
4	21	35	21	27	20	32	21	29
5	22	34	21	26	20	33	22	29
Mean	21	36	21	27	20	33	21	30
sd	0.4	1.9	0.0	1.2	0.5	0.5	0.5	1.3
CV (%)	2.1	5.5	0.0	4.5	2.7	1.7	2.6	4.4

sd standard deviation
CV Coefficient of Variation = (sd/mean) x 100

APPENDIX A

Methods of sample collection and analysis for T-7499

SAMPLE COLLECTION

Chamber concentration

All samples were taken from a sampling port at Level 2 of the exposure chamber. The first exposure was manually sampled by withdrawing chamber atmosphere from a sampling port on the exposure chamber into a polypropylene syringe fitted with a valved seal. For the second and subsequent exposures, an automated sampling system was used to transfer samples *via* PTFE tubing to a gas chromatograph. Automated samples of atmosphere from the chambers were allowed to purge the sampling line for 12 minutes before a sample was injected.

METHOD OF ANALYSIS

Chamber atmosphere samples were analysed by gas chromatography. The method of sample analysis is detailed, together with a summary of the method validation, in the Inhalation Analytical Procedure at the end of this appendix.

CALCULATIONS

GC analysis

The samples of chamber atmosphere were injected into a gas chromatograph, which was calibrated using vapour standards prepared in gas bags. The method for calculating the concentration of T-7499 from the mass used to prepare each vapour standard is given below in equations 1 and 2.

$$\text{Concentration} = \frac{V}{V_a + V} \times 1{,}000{,}000 \text{ ppm} \qquad (1)$$

$$V = \frac{W \times R \times T}{M} \times \frac{760 \text{ mm Hg}}{Atm} \qquad (2)$$

where V = gaseous volume of T-7499 (ml)
W = mass of T-7499 (mg)
M = molecular weight of PBSF (302.09 g/mole)
R = 0.08205 ml.atm/mmol.K
T = temperature (K)
Atm = atmospheric pressure (mmHg)
V_a = volume of air (ml)

In order to minimise the cumulative errors, which result from repeated rounding of numbers, much of the data in this report have been calculated continuously using unrounded numbers and only rounded for printing. Consequently, any further calculation using these rounded numbers will include rounding errors in the last significant figure, possibly leading to small apparent discrepancies with other data in the report.

APPENDIX A

(Methods of sample collection and analysis for T-7499 – continued)

COMPOUND SPECIFIC INHALATION ANALYTICAL PROCEDURE FOR T-7499

The analysis of T-7499 in air sample substrate

The method outlined in this document has been validated and is considered fit for the purpose of monitoring test atmospheres and blood headspace in an Inhalation Toxicology study.

This document details the basic procedures for the analysis of T-7499 sampled by syringe from test atmospheres. The resulting samples, of approximate concentration 100 to 500ppm, are analysed by GC. Study specific amendments and additions will be detailed within a supplementary document.

NOTE Throughout this document, the symbol **§** indicates that the relevant information is not available at present, but will be included in a Study specific supplement.

EFFECTIVE DATE:	**22 May 2000**

Test substance

T-7499 is mainly perfluorobutyl sulfonyl fluoride (PBSF), which has the formula $C_4F_{10}O_2S$.

Appearance	liquid
Subsample Storage of Test Mixture	A temperature of +4°C. Protected from moisture.

Equipment

Balance	Sartorius	BP4100
Syringes	Hamilton Hamilton	1000 series gas-tight (100 and 25 ml) 500 series gas-tight (500 ml)
Gas sample bags	SKC INC	Tedlar® 232-series (1 and 3 dm^3 capacity)
Syringe valve	Mininert	Push button valve
Vacuum pump	AEG	ADEB 56 (or equivalent)
Flow meter	J & W Scientific	ADM1000 (acoustic displacement)
General laboratory glassware		

Consumables

Syringes	Sigma Aldrich	20 ml polypropylene

APPENDIX A

(Methods of sample collection and analysis for T-7499 – continued)

Preparation of samples for analysis

Samples of the test atmosphere are collected using a 20 ml syringe fitted with a "Mininert" valve. The syringe needle is inserted into the sampling port and 10 ml of test atmosphere is withdrawn into the syringe. The valve is closed and the syringe removed from the sampling port for injection onto the GC.

The gas sampling valve of the GC is set to the "load" position and the syringe is placed into the injection port of the valve. The valve of the syringe is opened and the contents of the syringe are passed into the sampling valve. The valve is then switched to the "inject" position and simultaneously the run start is activated.

Preparation of calibration standards

Standards are prepared using the following method. The actual standard concentration ranges used are as detailed in the study specific supplement.

A volume of T-7499 is dispensed from the cylinder into a scintillation vial. Weigh approximately 670µg of T-7499 (400µl) and inject into a gas bag, mix thoroughly and make up to set volume with air to provide standard S1. The T-7499 is vaporised by gentle warming using a hot air blower.

Evacuate a series of gas sample bags of appropriate capacity and introduce by syringe measured volumes of air. Using gas tight syringe(s) fitted with a sealing valve, accurately dispense measured volume(s) of the T-7499 vapour into the gas sample bags *via* the injection port to produce standards covering the concentration range described in the study specific supplement.

Storage of standards and samples

The maximum storage periods for the various sample types are detailed below

Sample type	Storage conditions	Storage period
Gas standards	Room temp., light	5 days
Syringe samples	Room temp., ambient	150 minutes

APPENDIX A

(Methods of sample collection and analysis for T-7499 – continued)

Quality assurance measures

When the method is established on a chromatographic system six injections of a standard will be used to verify performance of the system. The parameters and acceptance criteria are set out below;

Parameter	Typical value	Acceptable limits
Plate count (USP)	3624	> 80%
Tailing factor (USP)	1.0958	± 20%
Repeatability (CV, n=6)	<1.4%	<5%
QC tolerance	< ±2%	< ±5%
QC tolerance at LOQ	< ±5%	< ±10%

The highest calibration standard will be compared against a standard of similar concentration prepared independently. The ratio of response factors will be acceptable if within the range 0.95 to 1.05.

A quality check standard must follow every 6 concentration samples for the analysis to be regarded as valid. The results of the quality check standards must lie within the QC tolerance limits.

A quality check standard of low concentration will be run to verify the LOQ for the run. The LOQ for the run will be regarded as the concentration of the lowest acceptable quality check standard.

Summary of method validation

The raw data for the method validation is located in study MIN/244.

Comparison of test blanks, standards and test samples showed that the analyte was well resolved from any potential interfering peak.

Precision data showed coefficients of variation for PBSF of less than 1.4% with standards in the range of 10,000 to 500 ppm and less than 2.0% to standards to 100ppm.

Least squares regression analysis, with a 1/c weighted linear weighting, for a peak area response against concentration of standard (100 to 10,000 ppm) produced a correlation coefficient of 0.999991 and relative errors less than 2.1% in the range 10,000 to 100 ppm. The Limit of Quantification (LOQ) for T-7499 will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 59.45 and 17.83 ppm respectively (calculated statistically using the standard deviation obtained for a solution of concentration 100 ppm).

Standards of T-7499 in the range 100 to 10,000 ppm stored at room temperature for 5 days and subsequently analysed against fresh standards showed concentrations within 5% of their nominal.

Samples of a standard (ca 1,000 ppm) of T-7499 stored in the injection syringe for 150 minutes under ambient conditions (room temperature under normal lighting conditions) and subsequently analysed against freshly injected standards showed concentrations within 5% of their nominal concentrations.

APPENDIX A

(Methods of sample collection and analysis for T-7499 – continued)

GAS CHROMATOGRAPHS IN INHALATION TOXICOLOGY AT 25 SEPTEMBER 1997

System No.	Components of gas chromatography system		
1	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated gas sampling valve, ECD and FID.
	Hewlett Packard	18593B	}
	Hewlett Packard	18596CX	} 7673 Autosampler
	Hewlett Packard	G1512AX	}
	ThermoQuest♣	SP4500	A/D interface
	ThermoQuest	PC1000	Integration software
2	Pye Unicam	PU4550	Chromatograph with gas valve and FID.
	Pye Unicam	PU4700	Autosampler
	ThermoQuest	SP4500	A/D interface
	ThermoQuest	PC1000	Integration software
3	Shimadzu	GC-14A	Chromatograph with FID.
	Shimadzu	AOC-1400	Autosampler
	Shimadzu	AOC-14	Autoinjector
	ThermoQuest	SP4500	A/D interface
	ThermoQuest	PC1000	Integration software
4	Pye Unicam	304	Chromatograph with FID.
	Pye Unicam	PU4700	Autosampler
	ThermoQuest	SP4400	Integrator
5	Pye Unicam	304	Chromatograph with FID.
	Pye Unicam	PU4700	Autosampler
	ThermoQuest	SP4400	Integrator
6	Shimadzu	GC-14A	Chromatograph with FID.
	Shimadzu	MGS-4	Automated gas valve
	Shimadzu	CR4-A	Integrator
7	Shimadzu	GC-14A	Chromatograph with FID.
	Shimadzu	MGS-4	Automated gas valve
	Shimadzu	CR4-A	Integrator
8	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated automatic gas sampling valve and FID.
	Hewlett Packard	G1513A	}
	Hewlett Packard	18596CX	} 6890 Series Autosampler
	Hewlett Packard	G1512AX	}
	ThermoQuest	SP4500	A/D interface
	ThermoQuest	PC1000	Integration software
9	Perkin Elmer	Autosystem XL	Chromatograph with programmable capillary inlet, heated automatic gas sampling valve and FID.

♣ formerly Spectra-Physics

APPENDIX A

(Methods of sample collection and analysis for T-7499 – continued)

MIN/244 - STUDY SPECIFIC SUPPLEMENT TO THE INHALATION ANALYTICAL PROCEDURE FOR T-7499

This supplement details additions and amendments to the procedure to be used for the GC assay of T-7499 obtained from air samples collected on the above study.

The assay, incorporating the additions and amendments, is suitable for the analysis of T-7499, in air, at concentrations within the range of 100 to 10,000ppm.

Details given in this supplement supersede those in the compound specific IAP.

EFFECTIVE DATE :	**3 July 2000**

Analytical standard

Name	T-7499, Perfluorobutyl sulfonyl fluoride (PBSF)
Batch number	FCS00001822
Purity	96-98% (Perfluorosulfolane 2-4%)
Expiry date	Not Stated
Supplier	Sponsor (filled by Manchester Tank)

Preparation of standards

Prepare standards in the nominal range 100 to 20,000ppm.

Calibration and Quantification

A 1/c weighted linear regression using 3 analytical standards over the range 100 to 1000ppm was used as the calibration before analysis commenced.

Summary of method validation

A different regression analysis was conducted on the 13 June 2000 for the initial acute study (MIN/244). The details are given below.

Precision data showed coefficients of variation for T-7499 of less than 1.6% with standards in the range of 10,000 to 500 ppm and less than 3.4% to standards to 100ppm.

Least squares regression analysis, with a $1/c^2$ weighted linear regression, for a peak area response against concentration of standard (100 to 10,000 ppm) produced a correlation coefficient of 0.999984 and relative errors less than 2.6% in the range 10,000 to 100 ppm. The Limit of Quantification (LOQ) for T-7499 will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 40 and 12ppm respectively (calculated statistically using the standard deviation obtained for a solution of concentration 100 ppm).

The change in regression analysis was due to a decrease in the baseline noise value produced from the integration. This resulted in a slight increase in peak areas at low concentration and had the effect of modifying and improving the accuracy of the results obtained.

APPENDIX A

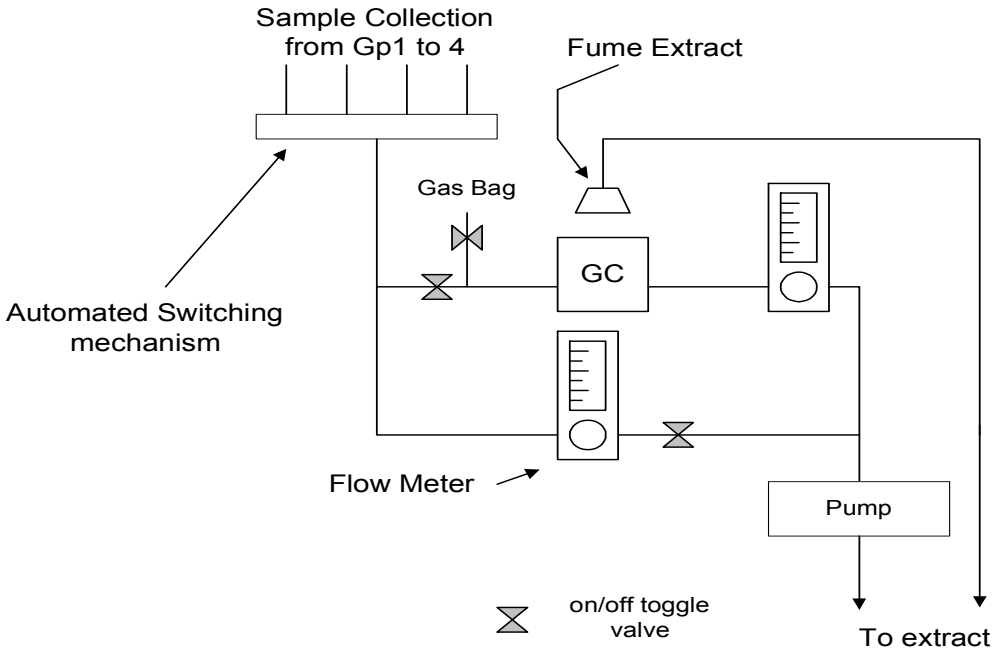
(Methods of sample collection and analysis for T-7499 – continued)

Chromatographs

The analysis is performed using chromatograph 2 in Y14.

Detector Temperature was increased from 100°C to 150°C.
Detector Range = 0
Carrier Gas (Helium = 2.75ml/min)
Split Vent (Helium = 24.5ml/min)
Split Ratio (1:8.9)
PBSF retention time = 2.74minutes

Sampling System



The mechanism for sampling from the chambers has been redesigned to give an automated system. The automated sampling system runs in sequence from high dose to control on a 60 minutes cycling time (15 minutes for each chamber). 12 minutes into each sample period per chamber, an additional timer provides a 250msec on/off contact closure to act as a remote start for the GC. The flow meters are calibrated in such a fashion that most of the sample extract proceeds through the excess sampling line with only a limited flow proceeding through the GC line. The Teflon tubing to the GC from the T-piece is 1/8" OD tubing, the remainder is ¼". The flow rates in the GC sampling and excess lines were 0.11 and 1.3L/min respectively.

APPENDIX A

(Methods of sample collection and analysis for T-7499 – continued)

The alternative method of analysis was by the drawing of a sample into a 20 ml polypropylene syringe from a sampling port on the exposure chamber. The syringe was flushed with the test atmosphere prior to sampling. The pressure within the syringe was allowed to equilibrate before the gas tight valve was closed and the syringe removed.

The calibration for the T-7499 test compound is conducted in the same manner as before.

EFFECTIVE DATE :	**14 July 2000**

Calibration and Quantification

A 1/c weighted linear regression using analytical standards over the range 100 to 500ppm was used as the calibration before analysis commenced. Quality Assurance check standards were run at 2 levels, 100 and 500ppm.

APPENDIX B

Chamber concentrations of T-7499 – individual sample values

Exposure No.	Sample No	Chamber concentration (ppm)		
		Group 2 (Low dose)	Group 3 (Intermediate dose)	Group 4 (High dose)
1	1[a]	202	364	486
	2[a]	198	361	544
	3[a]	202	358	485
	4[a]	213	361	459
	5[a]	191	356	504
	6[a]	196	363	486
	Mean	200	360	494
	sd	7.3	3.2	28.3
2	7	176	326	464
	8	200	351	479
	9	203	330	478
	10	173	[428][b]	498
	11	214	380	515
	12	220	12[c]	524
	Mean	198	280	493
	sd	19.3	151.3	23.4
3	13	207	373	486
	14	217	370	[424][b]
	15	207	346	508
	16	207	360	509
	17	209	385	500
	18	206	372	511
	Mean	209	368	503
	sd	4.0	13.4	10.2
4	19	204	374	521
	20	204	350	[330][b]
	21	193	383	550
	22	213	379	531
	23	205	382	540
	24	[60][d]	375	603
	Mean	204	374	549
	sd	7.2	12.2	32.1
5	25[a]	215	365	524
	26	196	334	488
	27	198	328	467
	28	195	356	488
	29	200	335	493
	30	217	350	506
	Mean	203	344	495
	sd	9.8	14.5	19.1

sd Standard deviation
[ ] Value excluded from calculation of mean and sd.

[a] Samples 1-6 and 25 were injected manually.
[b] Syringe changed during automated sampling.
[c] Syringe running low.
[d] Last sample taken after exposure period, due to late start of automated sampling system.